The epithelial–mesenchymal plasticity landscape: principles of design and mechanism of regulation

Jef Haerinck[1,2], Steven Goossens[2,3] & Geert Berx[1,2†]

[1]Molecular and Cellular Oncology Laboratory, Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

[2]Cancer Research Institute Ghent (CRIG), Ghent, Belgium

[3]Unit for Translational Research in Oncology, Department of Diagnostic Sciences, Ghent University, Ghent, Belgium

[†]e-mail: geert.berx@ugent.be

Abstract

Epithelial–mesenchymal plasticity (EMP) enables cells to interconvert between several states across the epithelial–mesenchymal landscape, thereby acquiring hybrid epithelial/mesenchymal phenotypic features. This plasticity is crucial for embryonic development and wound healing, but also underlies the acquisition of several malignant traits during cancer progression. Recent research using systems biology and single-cell profiling methods has provided novel insights into the main forces that shape EMP, which include the microenvironment, lineage specification and cell identity, and the genome. Additionally, key roles have emerged for hysteresis (cell memory) and cellular noise, which can drive stochastic transitions between cell states. Here, we review these forces and the distinct but interwoven layers of regulatory control that stabilize EMP states or facilitate epithelial–mesenchymal transitions (EMTs) and discuss the therapeutic potential of manipulating the EMP landscape.

Introduction

Throughout the development of multicellular organisms, each cell lineage increasingly sacrifices its differentiation potential for the actuality of a specific role — a specific set of functions supported by a stable cell identity. During this development, each cell fate decision adds additional constraints, locking cells into distinct lineage trajectories. Famously, this process was represented visually by Waddington as a ball rolling down a rugged landscape until, ultimately, coming to a halt in one of the lower valleys[1]. Under homeostatic conditions, most mature cell types remain locked in this stable and mature 'attractor' state. However, in response to microenvironmental stimuli or pathology, some cell types can regain plasticity within the confines of their identity. For example, many epithelial cells continue to inhabit a limited 'landscape of plasticity' consisting of multiple metastable and interconvertible phenotypic states[2-4].

Epithelial–mesenchymal transition (EMT) describes a shift from an epithelial to a mesenchymal cellular phenotype (Fig. 1). Typical epithelial characteristics, such as an apical–basal polarity, attachment to the basement membrane, and strong cell–cell adhesion, result in immobile cells, stably anchored in tightly linked epithelial sheets, which are wellsuited to act as barriers against the external or internal environment. During an EMT programme, the firm epithelial cell–cell adhesion is lost, matrix interactions are modulated, and the apical–basal polarity is exchanged for a front–back polarity. As a result, individualized and migratory mesenchymal-like cells, which often display invasive capacities, emerge from the epithelial sheets (Fig. 1a). These mesenchymal-like cells can reclaim their epithelial phenotype by undergoing a mesenchymal–epithelial transition (MET)[2-4].

EMT does not refer to a single process but, rather, encompasses a set of considerably different biological processes with overlapping characteristics (Fig. 1b). EMT was initially described in embryology[5,6]; the many morphogenetic movements during early embryonic development (type 1), whereby cells often undergo consecutive rounds of EMT and MET, are considered the motor of gastrulation and organogenesis[6]. Soon after, similar morphological and molecular changes were described in adult life, where EMT not only plays important roles in wound healing, tissue regeneration and the pathogenesis of organ fibrosis (type 2), but also contributes to cancer progression (type 3)[2-4,7].

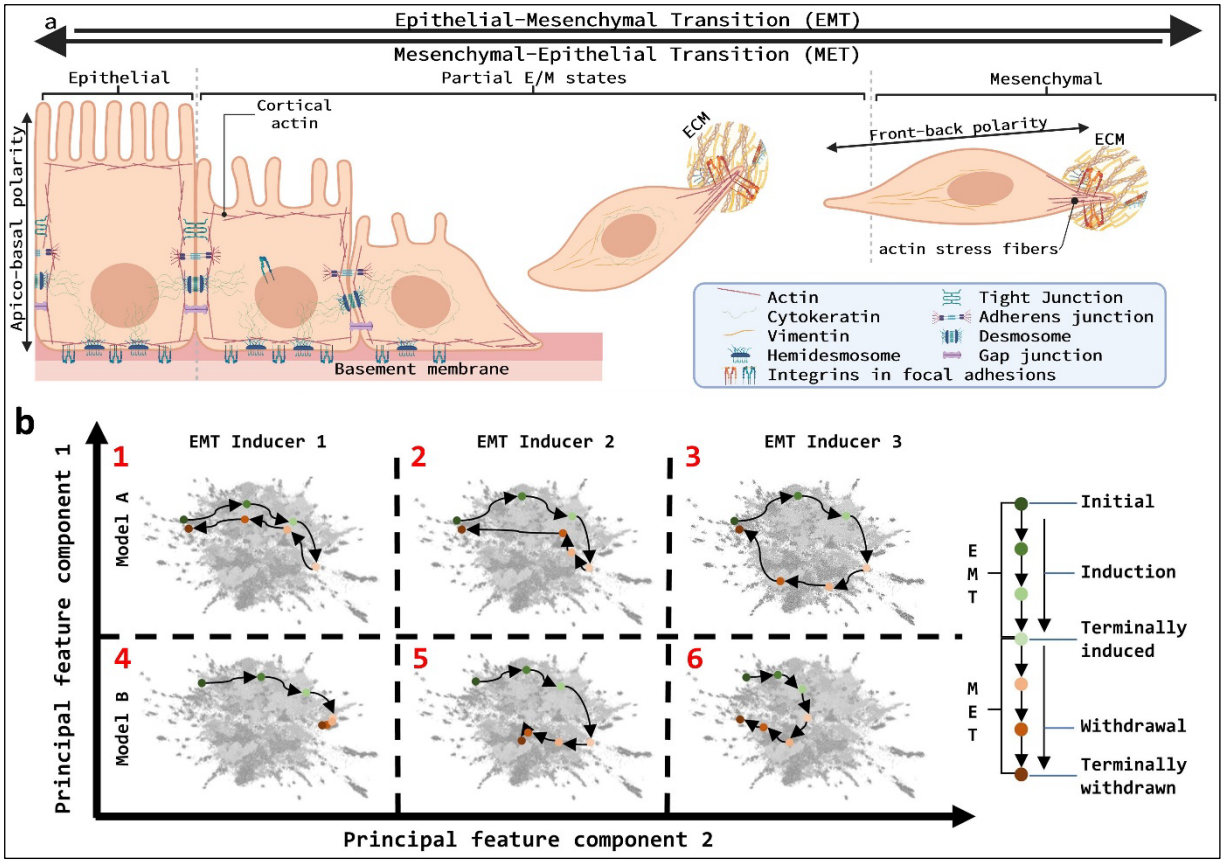


Fig. 1|Epithelial cells inhabit a landscape of plasticity

a| Epithelial cells mechanically integrate through adhesive interactions mediated by tight junctions, adherens junctions, desmosomes, and gap junctions. Furthermore, they attach to the basement membrane with hemidesmosomes and focal adhesions. As a result, epithelial cells are static, non-invasive, and arranged in tightly linked cell sheets. During epithelial-mesenchymal transition (EMT), epithelial markers such as cell-cell adhesion molecules and cytokeratins are downregulated, whereas mesenchymal markers such as N-cadherin, vimentin and fibronectin are upregulated. EMT is also coupled to a downregulation of epithelial integrins (for example, α6β4) and upregulation of mesenchymal integrins (for example, α5β1, α1β1, α2β1, αvβ3, and αvβ6)[350]. Through incomplete EMT processes, cells may inhabit intermediate states. Such partial EMT (pEMT) or hybrid EMT states simultaneously display epithelial and mesenchymal (E/M) properties. Following EMT, the resulting mesenchymal cells show front-back polarity and a cytoskeleton containing actin stress fibres. These cells show weak (or no) cell-cell adhesion, and they adhere to the extracellular matrix (ECM) through integrin-based focal adhesions. Consequently, EMT results in individual, motile cells with fibroblast-like morphology and invasive capacity[2-4,8]. **b|** Epithelial-mesenchymal plasticity (EMP) varies highly between contexts[9], being dependent on the system studied as well as the applied EMT driver. The diversity of EMP programmes implies countless EMP states, and argues against the often presented, one-dimensional, linear conception of the EMP spectrum (as in p a)[10]**.** Therefore, the EMP landscape is visualized here as a feature space and several potential EMT and mesenchymal-epithelial transition (MET) trajectories are plotted. Terminal states represent states in which cells remain indefinitely during continuous, intense exposure to, or long-term, complete withdrawal of, an EMT driver. Intermediate induction states can be thought of as partial responses to the stimulus as a result of the stimulus being applied only recently (time-course analysis) or at lower intensity (dose-response analysis), and vice versa for intermediate withdrawal stages. EMT and MET were classically described as symmetrical processes, the MET trajectory being the reverse of the EMT trajectory (panel 1). However, considering phenomena such as cell memory, MET may display distinct kinetics (panel 2) and/or distinct trajectories (panel 3) from EMT. Additionally, an EMT process may be completely (panel 4) or partially (panel 5) irreversible upon withdrawal of the stimulus which evoked it. Lastly, stimuli that evoke EMT in one system, may evoke only partial EMT (panel 6), or no EMT at all, or inhibit EMT, in other systems.

EMT and MET were originally considered binary processes: a transit from the epithelial to the mesenchymal phenotype and vice versa. However, recent single-cell RNA[9,11-18] and protein[18-20] profiling has demonstrated that cells can linger in a series of intermediate states along the epithelial–mesenchymal axis[21]. Such partial EMT (pEMT) states simultaneously exhibit epithelial and mesenchymal properties. Depending on the context, cells with hybrid E/M phenotypes can be either firmly locked[22,23] or free to roam throughout a vast landscape of alternative states — a phenomenon termed epithelial–mesenchymal plasticity (EMP)[4] (Fig. 1b).

This Review gives a concise overview of the general forces shaping the EMP landscape and the mechanisms that govern transitions therein. We First outline five distinct factors that contribute to shaping EMP; we review the well-appreciated roles of the microenvironment, lineage specification, and genetics, and then reflect on the emerging roles of hysteresis (cell memory) and noise-driven stochastic state transitions. Next, we discuss the specific regulatory mechanisms — transcriptional, translational, epigenetic and metabolic rewiring — that either stabilize specific cell states or drive state transitions within the EMP landscape.

Shaping the EMP landscape

EMP as a response to the local microenvironment

EMT is not a global phenomenon but, rather, a focal occurrence[3]. Each cell is embedded in and shaped by a microenvironment consisting of cellular neighbours, extracellular matrix and cytokine gradients. Only by sensing the changes in the microenvironment will epithelial cells know when and how to initiate EMT. Ligands such as TGF-β[24,25], HGF[26], FGF[27], EGF, Wnt[28] and Notch[29], for example, have been shown to induce or enforce EMT. Other microenvironmental parameters such as hypoxia[30,31], access to nutrients[32-34], shear forces[35-37] and matrix rigidity[38,39] also mediate EMP. Importantly, these parameters have mostly been studied under varying artificial *in vitro* conditions with fully transformed and genetically instable cancer cell lines. Nevertheless, these studies have illustrated the drastic influence of the microenvironment in determining the permissiveness of a cell for EMP, or in driving EMP itself. Microenvironmental influences may work synergistically or antagonistically; however, the many intricacies of these interactions remain to be elucidated.

An asymmetry arises when comparing the interactions of epithelial versus mesenchymal cells with the microenvironment. Epithelial cells, being held in place by firm adhesive properties, are 'overcome' by their microenvironment. By contrast, migratory mesenchymal cells, coordinated by chemotactic signals, actively seek out a specific environment. Such chemotactic mechanisms are crucial for body plan formation during development but also contribute to organotropism during cancer cell metastasis[40]. Cells in a partial EMT state may combine motility with some stability in their environment by migrating in a cluster-like fashion. In this case, the migratory unit contains a 'travelling microenvironment'. Besides tumour cells, these clusters can contain platelets[41], immune cells[42-45], cancer-associated fibroblasts[42,46] and extracellular matrix[42,47]. These clusters can stabilize the partial EMT state by maintaining TGFβ signalling[42]. Additionally, clustering can prevent anoikis[48,49] (a type of programmed cell death that occurs in anchorage-dependent cells upon loss of attachment to the extracellular matrix) and shield tumour cells from immune attacks, shear forces and other external stressors[50]. When it comes to metastasis, clusters are particularly effective at seeding secondary tumours[51-53].

Lineage specification and cell identity guide plasticity

Epithelial cell identity dictates the tendency for EMP. Epithelial cell subtypes, although similar in function, differ in shape, size and multicellular organization. Furthermore, these subtypes vary in their transcriptional and chromatin landscapes, as well as their expression of receptors and rate-limiting downstream mediators, all of which can have an impact on EMP. Additionally, functional[54] and histological[55] resemblance between cancer cells and developing embryos was recognized over a century ago. It has since been hypothesized that cancer cells acquire malignant traits through reactivation of dormant developmental programmes[56,57]. As such, EMP can, in many cases, be regarded as the result of fully differentiated, mature cells revisiting previously travelled paths in their developmental landscape[57-59].

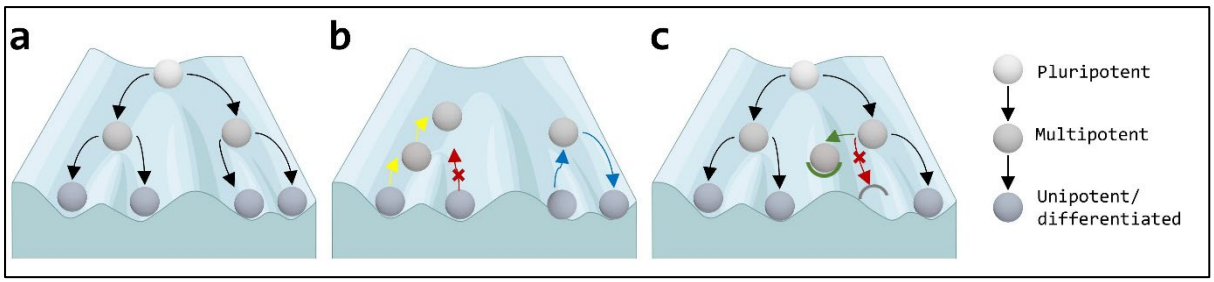


Fig. 2| The developmental landscape guides EMP.
a| During development, totipotent progenitors undergo consecutive cell fate decisions resulting in separate lineage trajectories. This process was visually depicted as a sphere rolling down a rugged landscape by C.H. Waddington[1]. Higher positions on the hill represent increased differentiation potency, and lower stability. Following a series of bifurcations (cell fate decisions) the cell reaches a stable and mature attractor state, represented by a valley. The lineage specification process and the cell's identity continue to impact its plasticity. **b|** Epithelial-mesenchymal plasticity (EMP) can promote dedifferentiation and endow cells with increased stemness. Several reports give weight to the perspective that this is the result of cells revisiting previously travelled paths in their developmental landscape during EMP (yellow arrow)[60-66]. The distinct developmental landscape of one lineage compared with another may greatly facilitate (yellow arrow) or obstruct (red arrow with red 'x') EMP[66]. Following dedifferentiation as a result of EMP, cells may redifferentiate towards another lineage (blue arrow). In this case, EMP allows cells to travel developmental paths of other lineages. **c|** Genomic changes may result in an alternative developmental landscape, obstructing access to developmental states (arrow with red 'x'), and/or stabilizing otherwise unoptimized attractors (green arrow). In this case, malignant EMP is the result of cells moving through alternative, unoptimized trajectories in Waddington's landscape. As such, germ-line mutations in EMT-associated transcription factors (EMT-TFs) result in aberrant embryonic development and somatic mutations acquired during tumour development may stabilize states with increased metastatic potential[22]. Similarly, epigenetic silencing of lineage-specific transcription factors during tumour progression may impede full differentiation.

Studies in support of such lineage-specific malignant progression have been accumulating[60-66]. Metastatic prostate cancer cells show reactivation of a developmental, ZEB1-dependent epigenomic programme[61]. Even within a single tissue, the cell of origin has a major impact on EMP displayed within a tumour[65,66]. Similarly, tumours resulting from KRAS Gly12Asp and p53 mutations in hair follicle stem cells were shown to be much more prone to display EMP than tumours arising from the same mutations in the interfollicular epidermis[66]. This difference persisted when epithelial tumour cells of both compartments were implanted in the same microenvironment. Subsequent analysis showed that this difference in EMP potential can be explained by transcriptional and chromatin-level priming in the healthy founder cells[66]. Additionally, the degree of differentiation affects EMP. In mice, hepatic progenitor cells transduced with H-Ras and SV40LT gave rise to significantly more mesenchymal-like tumours compared with hepatoblasts or adult hepatocytes transduced with the same oncogenes[65]. Complementary, functional loss of lineage-specific transcription factors, through silencing or mutation, enables dedifferentiation and promotes EMP. For example, loss GATA3, which is required for luminal epithelial

cell commitment[67], drives EMT in breast[68-71] and bladder[72] cancer. Similarly, loss of ELF5[73-75], FOXA[76,77], or KLF4[78,79], key modulators in cell fate decisions and maintenance, promote EMT across cancers.

Similar to classical EMT, the endothelium, a mesoderm-derived, simple epithelium, can undergo an endothelial-to-mesenchymal transition (EndoMT). Here, microenvironmental stimulation results in the loss of typical endothelial markers and the acquisition of a mesenchymal or myofibroblastic phenotype[80]. Complete EndoMT has been proposed as an important mechanism in the pathogenesis of fibrotic disorders[81-83]. Additionally, recent single-cell transcriptomics upon myocardial injury has shown that certain endothelial cell populations may undergo a partial EndoMT before reverting to their original phenotype post infarction[84]. Such a transient, partial EndoMT facilitates regeneration of the vascular network, and the morphological changes that endothelial 'tip' cells undergo during sprouting angiogenesis to form novel vessels are similar to those displayed during 'classical' embryonic EMT. In prostate cancer, tumour-associated endothelial cells can drive osteoblastic bone metastasis through an endothelial-to-osteoblast transition[85], which constitutes a specific type of EndoMT. Similarly, endothelial-to-osteoblast, as well as endothelial-to-chondrocyte transitions have been proposed to drive fibrodysplasia ossificans progressiva[86], a rare genetic disorder caused by mutations in the ACVR1 gene that is characterized by ossification of skeletal muscle and connective tissue[86]. Importantly, although the phenotypic changes and regulatory mechanisms between EndoMT and EMT seem similar, the downstream genes controlling cell–cell adhesion, cytoskeletal organization, extracellular matrix depositions and chemotaxis vary drastically dependent on the epithelial cell identity.

The genome limits the phenome

The plastic and reversible nature of EMP processes immediately denies that epithelial to mesenchymal shifts are directed by genetic changes. Rather, these shifts are governed by the epigenetic, (post-)transcriptional and (post-)translational machineries[87]. However, phenotypic plasticity ultimately remains constrained within the possibilities of the genotype. Several examples illustrate how somatic or germline mutations affect EMT in health and disease.

During embryogenesis, EMT-associated transcription factors (EMT-TFs) conduct a well-orchestrated harmony of morphogenetic movement. As EMT is essential for

development, mutations that affect the functionality of core EMT networks result in drastic developmental disorders[88]; for example, mutations in the core EMT-TFs ZEB2, SNAI2 (also known as SLUG) and TWIST1 can cause Waardenburg syndrome[89], Mowat–Wilson syndrome[90] and Saethre–Chotzen syndrome[91,92], respectively.

During cancer progression, selection of clones with specific mutations and chromosomal aberrations leads to continuous tumour evolution. These genetic alterations conspire with the EMT regulatory mechanism, resulting in the survival of the fittest and most resistant cancer clones. RAS activation has been shown to induce EMT via receptor-mediated upregulation of the EMT-TFs SNAI1[93] (also known as SNAIL) and SNAI2[94] (also known as SLUG). p53 inhibits the oncogenic RAS circuit[95,96]. As such, loss of p53 and oncogenic Ras synergize during cancer progression to stimulate EMT[93-98]. As explained above, mutations in lineage-specific transcription factors can also promote EMP. Additionally, loss of other tumour suppressor genes, for example, *IDH1*[99,100], *IDH2*[99] and *RB1*[101], has been shown to promote epithelial plasticity directly or indirectly via the (immune) microenvironment[102].

Cystic fibrosis exemplifies how genetic perturbations can drive EMP through the microenvironment in adult pathologies. *CFTR* mutations directly drive TWIST1-induced EMT in the airway epithelium[103]. At the same time, the lung environment of patients with cystic fibrosis is characterized by persistent infections[104], chronic inflammation[105], hypoxia[106] and high levels of TGF-β[107], which can then further enhance epithelial plasticity[103]. Similarly, tumour mutations can both promote or inhibit immune infiltration[108]. In turn, changes in the cellular composition of the tumour micro-environment affects its permissiveness for EMP[102].

Additionally, copy-number variations can influence EMP; copy number gain of the EMT-TF gene *ZEB1* has been shown to promote EMP in prostate cancer[109]. Notably, loss of function of the protocadherin-encoding *FAT1*, one of the most frequently mutated genes across human cancers, stabilizes a hybrid EMT state that contributes to metastasis[22]. Whereas the previous section discussed how EMP can be the result of cells revisiting previously traversed paths in their developmental landscape (Fig. 2b), this section highlights how mutations can reshape the EMP landscape by facilitating access to unoptimized attractor states in Waddington's landscape (Fig. 2c).

Cellular history shapes the EMP landscape through EMT memory

EMT processes are governed by hysteretic control mechanisms[110] (Fig. 3). The term hysteresis describes phenomena in which the state of a system depends on its history and was first coined to describe the dependence of the magnetic moment on past changes in the magnetic field[111]. Similarly, how a cell reacts to EMT-inducing or MET-inducing stimuli not only depends on the current stimuli but also on the history of that cell, for example, previous stimuli acting on that cell, past differentiation and dedifferentiation trajectories and the time of residence within the current cell state. For that reason, hysteresis is sometimes referred to as 'cellular memory'. As with any traveller, cells 'wandering' through the EMP landscape are guided by their memories.

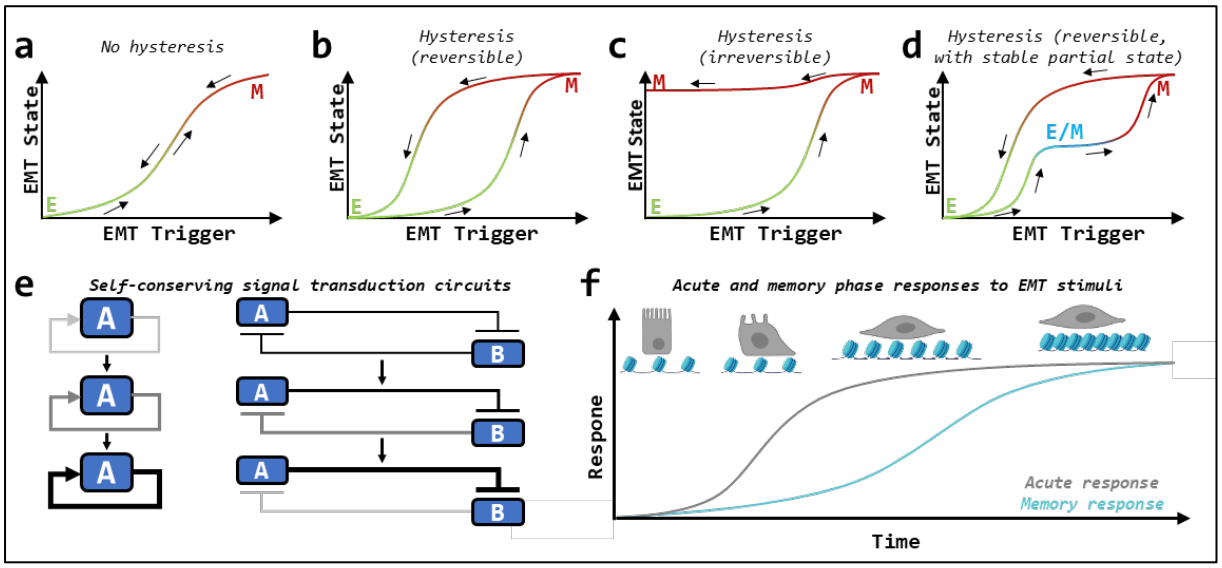


Fig. 3| EMT is controlled by hysteretic control mechanisms.
a| In the absence of hysteresis, the stimuli evoke a linear epithelial-mesenchymal transition (EMT) response and transitions between the epithelial and mesenchymal states are gradual. **b)** In the presence of hysteretic control mechanisms, EMT is characterized by non-linearity, and bistability or multi-stability. EMT and mesenchymal-epithelial transition (MET) trajectories do not overlap, but ,rather, form a hysteretic loop. **c|** If self-conserving feedback mechanisms are sufficiently strong, the mesenchymal phenotype can be maintained indefinitely, even after complete withdrawal for the stimuli which initially evoked it. **d|** As EMT and MET form distinct trajectories, it is possible for hybrid EMT states to be present in one direction, but not the other. **e|** Multi-stability in hysteretic systems is the result of self-sustaining signal-transduction circuits. In the simplest form, these are positive feedback loops (left) or double-negative feedback loops (right). **f|** Additionally, the acquisition of stable epigenetic marks, through DNA methylation, histone modification, or the incorporation of histone variants, contributes to EMT memory. These epigenetic changes can lag behind transcriptional and/or morphological changes. This delayed response on one level compared with another results in a two-tier mechanism in which an acute response, for example, transcriptional and/or morphological, is followed by a slower but more persistent memory response, for example, chromatin remodeling. As a result, the prolonged presence of an EMT stimulus after complete EMT may further stabilize the mesenchymal state and result in irreversible, or less reversible, EMT compared with shorter exposure.

Hysteresis underlies non-linear responses and bistability (or multi-stability) perceived across epithelial-mesenchymal axes (Fig. 3a-d). Put simply, the stimulus required to transition from the epithelial to the mesenchymal state is greater than the stimulus necessary to maintain the mesenchymal state once reached. Bistability depends on self-conserving signal-transduction circuits[112,113]. In their simplest form, these circuits can be positive feedback loops or double-negative feedback loops (Fig. 3e). If these feedback mechanisms are sufficiently strong, the cells may maintain their mesenchymal state indefinitely even upon complete withdrawal of the stimulus that initially evoked it[112] . Besides such self-reinforcing feedback loops, the acquisition of stable epigenetic marks, through DNA methylation or histone modification, contributes to EMT memory[114] (Fig. 3f).

Hysteresis implies that EMT and MET are asymmetric processes following distinct trajectories. Asymmetry between forward and backward processes is a key hallmark of hysteretic systems. This can be easily seen by plotting a dependent, EMT-marking variable as a function of an independent, EMT-inducing variable. On such a plot, forward and backward trajectories will not overlap, but rather form a so-called hysteresis loop (Fig. 3a-d). Time-course single-cell profiling supports this distinction between EMT and MET processes[9,19,115]. Indeed, studies show that mesenchymal cells can bypass some of the partial states transited during EMT and, instead, pass through a distinct MET state. Additionally, one study showed that of the three epithelial cell states present in untreated HCC827 lung cancer cells, only two were restored following consecutive EMT and MET[19]. Additionally, memory of EMT–MET cycles seems to have functional consequences, aiding metastasis[115,116] or augmenting plasticity and stem cell-like properties[117].

Noise-driven stochastic state transitions facilitate plasticity

Numerous cell lines display several EMP states *in vitro*[12,118-124]. Interestingly, sorted subpopulations and single cells often regenerate the phenotypic equilibrium of the parental culture[12,120-123], albeit with a clone-dependent phenotypic distribution[119,121]. How can such phenotypic heterogeneity spontaneously arise in the absence of genetic or extrinsic perturbation? Several mechanisms can explain the observed phenotypic equilibria in *in vitro* isogenic cultures. Paracrine feedback loops may continuously balance the fraction of cells within each state[125]. Additionally, pattern formation mechanisms, for example, GREM1/BMP2-mediated reaction-diffusion[126] or juxtracrine

Notch–Delta–Jagged signalling[127], can restore phenotypic heterogeneity. However, even in the absence of active mechanisms, phenotypic heterogeneity can be maintained. Several studies point to a role for noise in driving stochastic cell state transitions[122,128-131] (Fig. 4). If transition rates between phenotypic states are constant, the same phenotypic equilibrium will be restored regardless of the initial distribution as long as there is at least one direct or indirect path between any two states[122] (Fig. 4c).

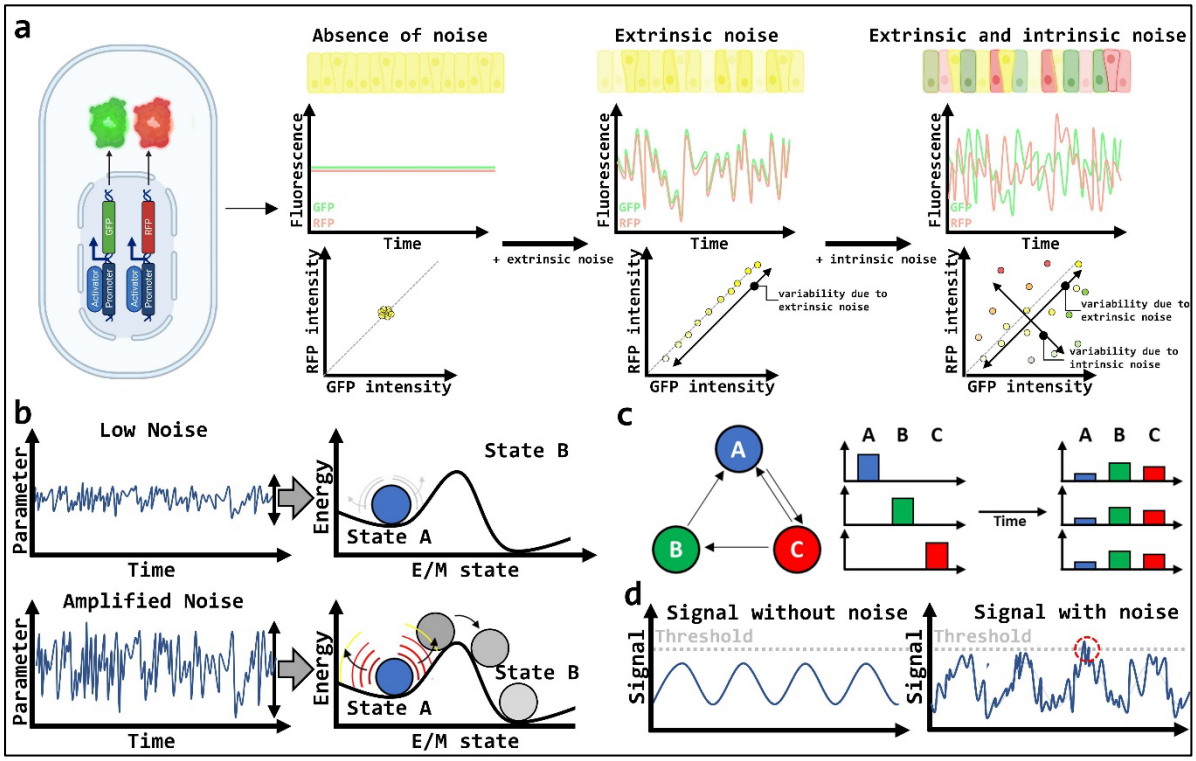


Fig. 4| Noise-driven stochastic state transitions enable spontaneous emergence of phenotypic heterogeneity

a| Cellular noise can be investigated using a dual-reporter system[130]. In this approach, two equivalent, independent reporter genes are placed in the same cell. In the absence of noise, expression of both reporters will correlate perfectly within cells and for each reporter, the same amount will be produced across cells. Extrinsic cellular noise, for example, through fluctuations in concentration, state or localization of biomolecules, and the random partitioning of biomolecules during cell division, will cause differences in expression between supposedly identical cells. Intrinsic cellular 'noise', which results from the inherent stochasticity in biochemical processes, will result in a loss of correlation between the reporters within cells. Part a adapted with permission from ref. 128, AAAS. **b|** In the absence of sufficient noise or external inputs, no cell state transitions take place (upper). Amplified noise facilitates cellular escape from local minima in the energy landscape, allowing them to roam the landscape of epithelial-mesenchymal plasticity (EMP) through stochastic state transitions (lower). **c|** If transition rates between multiple phenotypic states remain constant and if there is at least one direct or indirect path between any two states, equilibrium will be restored regardless of the phenotype distribution in the starting population, even in absence of active mechanisms. **d|** A weak sinusoidal signal is insufficient to reach the threshold required for activation of a biological process. Adding noise to this signal allows thresholding through so-called 'stochastic resonance'[132]. EMT, epithelial-mesenchymal transition.

Stochastic cell state transitions require: 1) a source of noise[133], 2) an amplification of this noise, which usually consists of small and transient fluctuations, in a manner to significantly affect the phenotype[129], and 3) mechanisms to stabilize the phenotype once reached[133]. Noise can be both cell-intrinsic or cell-extrinsic depending on its source[130] (Fig. 4a). Intrinsic noise results in differential expression of equivalent, independent reporters placed in the same cell[130], for example, because of the inherent stochasticity in biochemical processes. By contrast, extrinsic noise causes differential expression between cells in a population[130], which could be the result of fluctuations in the concentration, localization and general state of biomolecules. For example, the random partitioning of parent cell components across daughter cells during cell division is an important source of extrinsic noise that may drive EMT[128,129] or MET[134]. As such, random partitioning of SNAI1 has been predicted to contribute to the phenotypic equilibrium perceived in cultured PMC42-LA breast cancer cells[128]. In any case, noise of sufficient amplitude will activate the corresponding signalling cascades, amplifying its signal and causing phenotypic transition. The resulting phenotype can then be stabilized through hysteretic mechanisms, as described above.

Increased noise facilitates spontaneous cell state transitions[135,136]. Metastable states can be conceived as local minima in an energy landscape. The depth of each minimum corresponds to the stability of the cell state. In the absence of noise or other input, cells cannot vacate their local minimum. Sufficient noise allows cells to overcome the hill, or energy barrier, between minima[135,136] (Fig. 4b). As transitions from shallow to deep wells are less likely than the reverse, the relative stability of the different states determines the phenotypic equilibrium[136]. Additionally, noise may also facilitate state transitions that result from extrinsic signals. In a phenomenon termed stochastic resonance, noise can boost a signal that would otherwise be too weak to reach a change-inducing threshold (Fig. 4d)[132]. The main role of noise in the EMP landscape consists not of shaping the energy landscape itself but, rather of determining the transition rates between states.

Are noise-driven stochastic state transitions relevant to *in vivo* plasticity? One may argue that the influence of such noise decays in irrelevancy next to the more widely appreciated sources of phenotypic heterogeneity: genetic diversity and the microenvironment. However, spontaneous EMP in some cancer cell cultures partially recapitulates the phenotypic plasticity observed *in vivo*[12,137], supporting the relevance

of stochastic mechanisms. Secondly, noise plays a crucial role in developmental pattern formation[136,138,139] and cell-fate decisions[140,141]. Boundary formation during development happens not despite but, rather, due to noise-driven plasticity[136,138,139]. Thirdly, whereas noise implies randomness, the amplitude of said noise is strongly modulated by both evolutionary pressures as well as regulatory mechanisms[138,142-145] (Box 1). Only if the amplitude exceeds its optimal range does the resulting plasticity become disruptive to the organism[138]. Interestingly, the fast replication characteristic of cancer cells may increase stochasticity. Similarly, genomic damage increases cell-to-cell variation in gene expression[146]. Lastly, stochastic cell state transitions provide advantages in unpredictable environments when compared with responsive cell state transitions[147], for example, if cells do not possess the machinery to sense or respond to an external stressor or if cell survival requires transition to the resistant state prior to the environmental change[141,147,148]. As such, the distinction between stochastic and responsive transitions may prove essential in understanding the role of EMP in therapy resistance and immune escape.

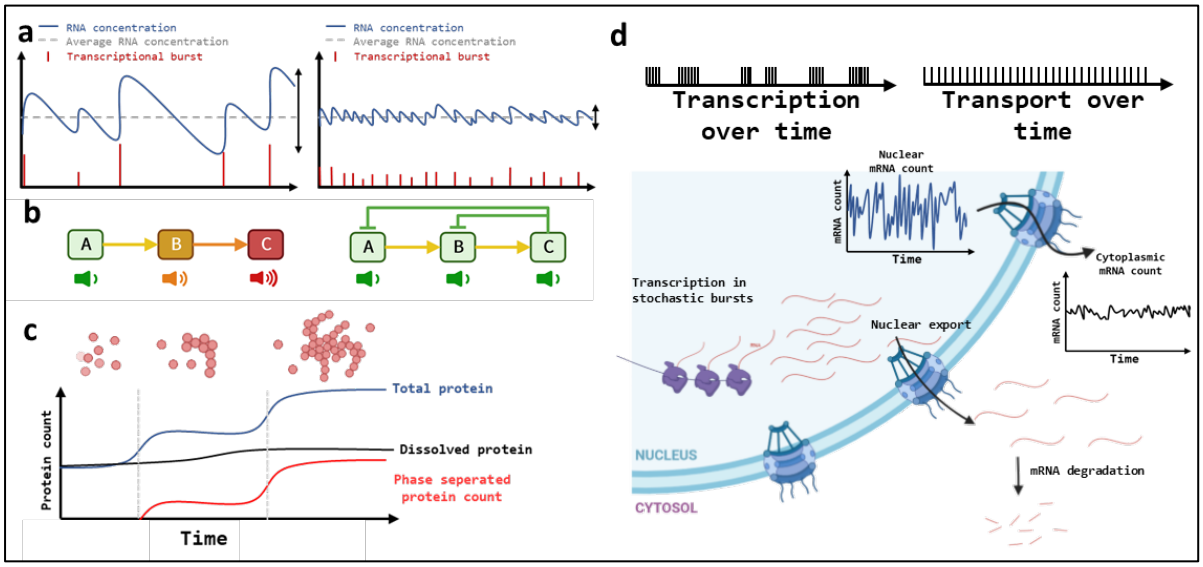
Box 1: the amplitude of cellular noise is regulated by both evolutionary pressures and regulatory mechanisms

Although noise implies randomness, the amplitude of said noise is strongly modulated by both evolutionary pressures, as well as by regulatory mechanisms[138,142-145]. As this may seem paradoxical, we selected some of the key mechanisms by which cells regulate cellular noise to illustrate this point.

Most genes are transcribed in transcriptional "bursts" — short periods of high transcriptional activity interleaved with long-lived periods in which no transcription takes place. This transcriptional bursting, which is thought to be caused by stochastic remodelling (opening and closing) of promoters[142], is a major source of gene expression noise. Bursting kinetics are highly gene-specific[149] and can be described in terms of burst frequency, and burst size (the average number of mRNAs produced during a single transcriptional burst). Besides transcriptional bursting, translational bursting may further contribute to gene expression variability.

Cellular noise is controlled through a multitude of mechanisms, of which we selected three general examples. Firstly, promoter architecture has a major impact on gene expression noise. The presence of a TATA box, enhancer distance, and enhancer strength are crucial determinants of the bursting kinetics[150]. Large, infrequent burst will produce large variability in mRNA levels, whereas small, frequent bursts of transcription will produce less noise (see the figure, part **a**). Secondly, gene regulatory network (GRN) architecture can promote response robustness by decreasing noise in gene expression and signal integration. For example, incorporation of negative feedback loops in GRNs allows autoregulation, resulting in decreased noise compared with purely linear cascades[151] (see the figure,

part **b**). Lastly, spatial compartmentalization enables more precise titration of biological components, filtering noise[152] (see the figure, parts **c,d**). Phase separation buffers noise[153] (see the figure, part **c**). Some biomolecules can undergo phase separation, creating membraneless compartments. By depleting excess biomolecule, one phase guards another from variability. For example, liquid-liquid phase separation[153] as well as cluster formation[154] were shown to reduce protein-level noise. Similarly, in aneuploid cells, protein aggregation was shown to function as a dosage-compensation mechanisms by depleting excess protein from the cytosol[155]. For biomolecules produced in one compartment but functional in another, concentration variability resulting from noisy production can be buffered by rate-limiting transport between those compartments. For example, transcription is occurs in bursts, resulting in noisy RNA expression within the nucleus. However, a rate-limiting transport step averages the introduction of RNAs in the cytoplasm over time, resulting in decreased concentration fluctuations[144] (see the figure, part **d**).



Regulatory mechanisms underlying EMP

Where the previous sections discussed the general forces governing the EMP landscape, the following part delineates more specifically the molecular mechanisms that underlie EMP states and EMTs (Fig. 5).

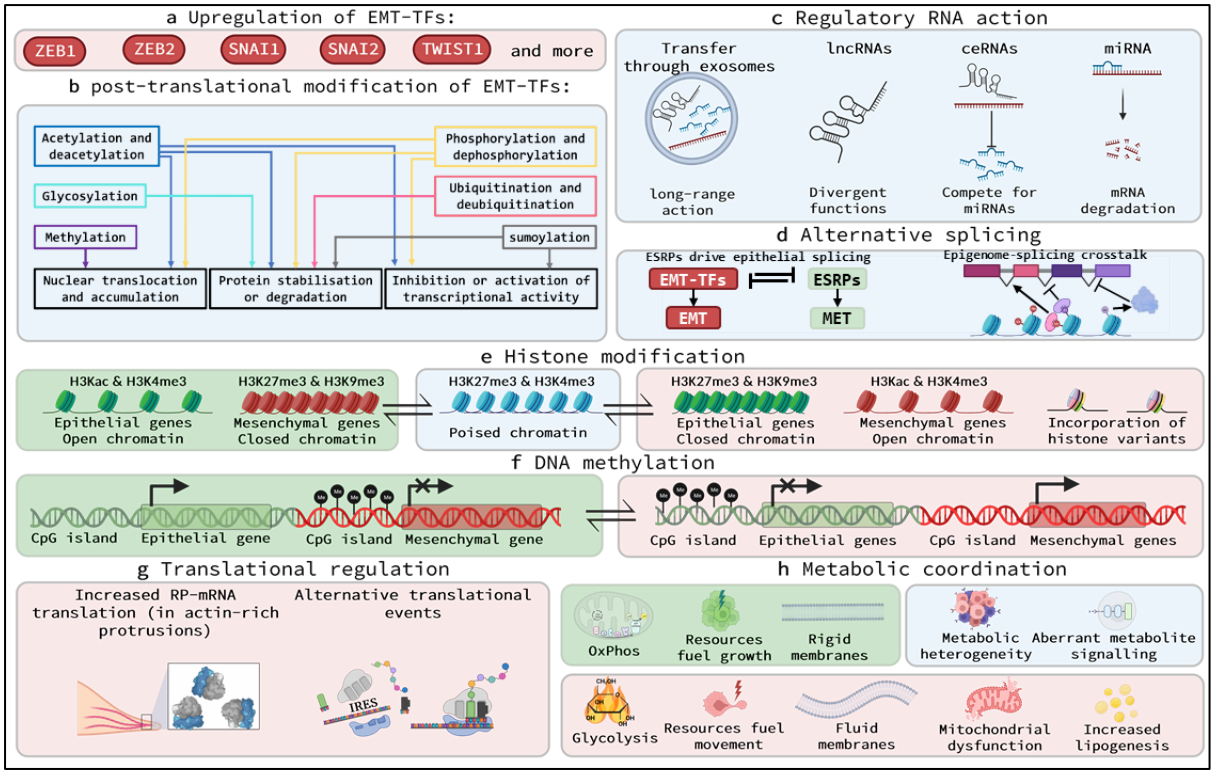


Fig. 5: Several levels of regulation control the epithelial-mesenchymal transition (EMT)

a| Epithelial-mesenchymal transition (EMT) is orchestrated by EMT-associated transcription factors (EMT-TFs). The core EMT-TFs include SNAI1/2, TWIST1 and ZEB1/2. **b|** The complex regulation of EMT-TFs occurs (in part) by post-translational modifications that affect the stability and degradation, the transcriptional activity and/or the nuclear translocation and accumulation of EMT-TFs[156]. **c|** Non-coding RNAs (ncRNAs) contribute to EMT and mesenchymal-epithelial transition (MET). MicroRNAs (miRNAs) silence mRNAs; for example, the *miR-200* family represses ZEB transcription factors, whereas the *miR-34* family represses SNAI1. Competitive endogenous RNAs (ceRNAs) can inhibit miRNA function by sequestering these miRNAs away from their targets[157,158]. Long non-coding RNAs (lncRNAs) combine base-pair complementation and RNA folding to enable interaction with both nucleic acids and proteins. LncRNAs modulate EMT on every level[159,160].Non-coding RNAs can travel long distances in circulation. **d|** Epithelial splicing regulatory proteins (ESRPs) are key regulators of epithelial splicing programmes[215]. As such, they can drive EMT as well as contribute to maintaining the epithelial cell state. Conversely, loss of ESRPs can drive an EMT. EMT-TFs inhibit and are inhibited by ESRPs. Epigenetic remodelling drives alternative splicing by slowing RNA polymerase II (RNA Pol II), or by specific histone modifications that recruit splicing factors, promoting the epithelial or mesenchymal phenotype. Consequently, crosstalk between the epigenome and the splicing machinery further modulates EMT[216,217]. **e|** In epithelial cells, epithelial genes show an open, accessible chromatin structure while mesenchymal genes are packed in closed, inaccessible chromatin. Following EMT, the opposite is true. Regardless of epithelial or mesenchymal state, H3K27ac and H3K4me3 marks are associated with open chromatin while H3K27me3 and H3K9me3 marks are associated with closed chromatin. Chromatin containing both the H3K27me3 and H3K4me3 marks is in a poised state and can be quickly activated or repressed on stimulation. **f|** In epithelial cells, there is hypomethylation of epithelial promotors and hypermethylation of mesenchymal promotors. Following EMT, the opposite is true. **g|** EMT is linked to an increase in ribosome biogenesis and protein synthesis. Additionally, the actin-rich protrusion that aid migration in mesenchymal cells were shown to be hot spots for ribosomal protein (RP)-mRNA translation, increasing overall protein synthesis. Alternative translational events, such as internal ribosome entry site (IRES)-dependent translation and N^6-methyladenosine (m6A) methylation of Snail mRNA, result in increased EMT-TF translation. **h|** Metabolic rewiring during EMT re-routes resources from proliferation to movement. EMT is associated with increased glycolysis and lipogenesis, but also mitochondrial dysfunction. Metabolic rewiring causes changes in bioactive metabolites which activate downstream signalling. Additionally, changes in lipid metabolism impact the cell membrane composition and, thus, its fluidity. Within a single tumour, cells may display diverse EMP

states whose functional characteristics are underpinned by distinct metabolic pathways. OxPhos, oxidative phosphorylation.

EMT-TFs: master regulators of EMP

EMT is regulated by EMT-TFs. Traditionally, five canonical EMT-TFs are recognized: ZEB1, ZEB2, SNAI1, SNAI2 and TWIST1[2-4] (Fig. 5a). Initially identified as key regulators during embryonic development[161-166], later studies showed their involvement in cancer progression[7,167-172] and fibrosis[173-176]. These EMT-TFs are key transcriptional regulators of EMT, acting as repressors of epithelial genes such as *CDH1* (which encodes Cadherin-1, also known as epithelial cadherin or E-cadherin) by binding the E-box motifs in their promoters[170,172,177,178]. Additionally, EMT-TFs can activate transcription of mesenchymal markers by forming complexes containing other DNA-binding units[179]. Besides these five core EMT-TFs, many additional EMT-TFs have been recognized including FOXC2[180], Homeobox protein goosecoid (GSC)[181], KLF8[182], PRRX[183,184], RUNX2[185], SIX1[186], TCF3 (also known as E47 or ITF1)[187] and TCF4 (also known as E2-2 or ITF2)[188].

Although forced expression of a single canonical EMT-TF is sufficient to induce a shift to a mesenchymal phenotype, EMT-TFs are not always redundant[189-191]. They show distinct spatiotemporal expression profiles during development and homeostasis[189-191]. Additionally, different EMT-TFs interact with distinct components of the epigenetic, transcriptional, and post-transcriptional machineries[189,192]. Moreover, complex regulation of the SNAI, TWIST and ZEB families of EMT-TFs by post-translational modifications, such as acetylation, glycosylation, methylation, phosphorylation, sumoylation and ubiquitination, directly affects the accumulation, degradation, stability, nuclear translocation or transcriptional activity of these transcription factors[156] (Fig. 5b). Furthermore, EMT-TFs have several pleiotropic functions that are, at least seemingly, unrelated to EMT[190] (discussed further in '*EMT-TFs beyond epithelial plasticity*').

Regulatory RNAs contribute to EMP regulation

The many instigators of EMT and MET processes seem to converge on a doublet of EMT-TF–microRNA (miRNA) double-negative feedback loops[3], which underlies the bistability of EMT (Fig. 6). These feedback loops concern the reciprocal inhibition between ZEB-family transcription factors and *miR-200*-family miRNAs[193,194], and the

mutual inhibitory loop between SNAI1 and *miR-34*[195,196]. Epithelial states display high *miR-200* and *miR-34* expression, whereas mesenchymal states show increased EMT-TF expression. Several competing models, for example, the ternary chimaera switch (TCS) model[197] (Fig. 6a) and the cascading bistable switches (CBS) model[198,199] (Fig. 6b), have been proposed to elucidate how this core regulatory hub governs phenotypic transitions. The key difference between these models is that self-activation of ZEB1 is included in the TCS but not the CBS model. Both models predict a tri-stable system consisting of an epithelial state, a mesenchymal state, and a hybrid E/M state (Fig. 6c-d). The TCS model postulates that such tri-stability is created solely by the ZEB1-*miR-200* loop, whereas the SNAI1-miR-34 loop acts as a monostable noise-buffering integrator[197]. By contrast, the CBS model proposes that both loops work as bistable switches. In that case, the SNAI1-*miR-34* loop guides the transition from the epithelial state to the hybrid E/M state. The ZEB1-*miR-200* switch then causes the transition to the final mesenchymal state[198].

Upregulation of EMT markers can precede *miR-200* downregulation[157,198]. This supposed anachronism can be explained through competitive endogenous RNA (ceRNA) action. ceRNAs compete for miRNAs, sequestering miRNAs from their targets[158]. As such, ceRNAs allow fast suppression of miRNA function. Indeed, integration of the 120 h miRNA half-life in the CBS model results in a bistable system consisting of an epithelial state and a hybrid E/M state[157,200]. Only upon integration of ceRNAs does transition towards the mesenchymal state become possible[157]. Integration of ceRNAs into EMP regulatory networks thus facilitates and accelerates state switching. The stoichiometry between competing miRNA response elements (MREs) and miRNAs is a critical parameter controlling both the stage of EMT and the reversibility of the EMT process. Indeed, the induction of a single highly expressed mRNA molecule, for example, *FN1*, has been shown to be sufficient to regulate EMT[157]. This dependence on stoichiometry also implies that the low miRNA environment found in some cancers facilitates ceRNA regulation[201,202].

Long non-coding RNAs (lncRNAs) are a third class of regulatory RNAs that govern EMT. Combining base-pair complementation and RNA folding, lncRNAs can interact with both nucleic acids and proteins to modulate EMT on every level. The catalogue of lncRNAs that modulate EMT processes, and their varied mechanisms of action, have recently been reviewed elsewhere[159,160].

The effect of regulatory RNAs is not limited to the cell in which they are transcribed. Rather, regulatory RNAs may be captured from the environment as the cargo of exosomal vesicles[203,204]. For example, exosomal miRNAs from hypoxic stromal cells were shown to promote EMT and metastasis in lung cancer[205]. Similarly, exosomal lncRNAs facilitate or supress EMT in pancreatic[206,207], gastric[208], lung[209] and bladder[210] cancer. Importantly, exosomes may travel long distances in circulation[203,204]. As a result, regulatory RNA action is not constrained to the microenvironment of the cells expressing the RNAs.

The emerging role of regulatory RNAs (Fig. 5c) demands caution when studying EMT through gene-perturbation approaches. Genes whose function is disrupted at the protein level may maintain regulatory control through transcript-level MREs. Inversely, overexpressing protein-coding sequences disregards *trans*-regulatory functions of introns and 5' or 3' untranslated regions (UTRs). A better understanding of the interplay between regulatory RNAs and EMT holds diagnostic and therapeutic potential. lncRNAs often show very specific tissue- or condition-specific expression. As such, they may form promising therapeutic targets for anti-EMT therapies. Additionally, as exosomal non-coding RNAs can be readily assessed in liquid biopsies, they hold promise as non-invasive biomarkers for disease progression.

DNA methylation and chromatin remodelling drive transitions and stabilize EMP states

The modification of both DNA and its packing units, for example, by DNA methylation, histone post-translational modifications (PTMs) or the incorporation of histone variants, is a crucial mechanism underlying EMT processes[192]. Some of these epigenetic marks are readily reversible, as is required for plasticity. Other marks are relatively stable[211], and thus contribute to hysteresis, or irreversible, such as epigenetic marks obtained during differentiation, which form the basis of cell identity.

Focal hypermethylation of the CDH1 promoter has long been recognized to be a hallmark of EMT[212-214]. Since then, more general trends have emerged. In general, EMT is associated with hypermethylation in the promoters of epithelial genes, and hypomethylation of mesenchymal genes and EMT-TFs (Fig. 5f). Additionally, key regulators of EMT, such as the EMT-TFs[215-217] or the *miR-200* family[218-221], regulate and are regulated by DNA methylation.

At the chromatin level, EMT is associated with a switch from more to less accessible chromatin in epithelial genes, and oppositely so for mesenchymal genes (Fig. 5e-f). Chromatin accessibility is strongly influenced by post-translational modifications on the histone H3 tail. Active chromatin is then mainly associated with H3K acetylation (H3Kac) and H3 trimethylated at K4 (H3K4me3)[218], whereas repressed chromatin bears H3K27me3, H3K9me3 and DNA methylation[192,218,221]. Chromatin bearing both H3K27me3 and H3K4me3 is in a poised state and can be rapidly repressed or activated upon stimulation[192]. Additionally, it was recently shown that the histone mark H3K36me2 underlies the mesenchymal state across a variety of contexts, whereas its erasure determines the epithelial state[222]. Apart from H3 modifications, acetylation of H2BK5 has been demonstrated to protect the epithelial phenotype in trophoblast stem cells as well as breast cancer cell lines[223].

Importantly, epigenetic reprogramming may also contribute to the execution of EMT by driving alternative pre-mRNA splicing (Fig. 5d). It is known that, expression changes in key splicing factors such as ESRP1/2 during EMT result in alternative splicing of different genes involved in functional aspects of EMT: polarity, migration and invasion and cytoskeleton organization[224]. Recently, however, it was shown that dynamic epigenetic changes in H3K27ac/me3 levels, as well as recruitment of HDAC1 by ZNF827 to coding genomic regions, are at the basis of regulating alternative splicing during epithelial plasticity changes. Additionally, these chromatin-induced splicing changes are sufficient to initiate EMT[225,226].

The incorporation of variant histones provides another mechanism through which epigenetic marks direct EMP[227,228]. EMT seems to cause a general decrease in canonical histones[227]. Additionally, specific histone variants seem to promote[227] or supress EMT[228]. Important to understand is that replacing a histone erases the post-translational modifications carried by that histone. As such, histone replacement contributes to the chromatin responsiveness essential for EMP.

Differences between chromatin landscapes of distinct epithelial cell types can explain how cell identity governs EMP modality. Indeed, in squamous cell carcinoma it was shown that the chromatin landscape of the cancer cell-of-origin strongly influences EMP propensity during further tumour development[66]. Similarly, reactivation of developmental epigenomic programmes was shown to underlie EMP and metastasis in prostate cancer[61]. These examples give weight to the long-held perspective that EMP is, in essence, the reactivation of developmental programmes.

Reversible, but persisting, epigenetic marks provide one mechanism for the establishment of EMT memory[114,229]. In cancer cell lines treated with therapeutic agents, DNA methylation was shown to be a causal factor underlying EMT-mediated therapy resistance[217,230]. Interestingly, such cell lines maintained their EMP state in the absence of therapeutic agents and only reverted to an epithelial state in the presence of demethylating agents. Such examples show that that DNA methylation plays an important role in the stabilization of certain EMP states. Additionally, an EMP state, once reached, may be stabilized by prolonged exposure to the stimuli that initially evoked it. Indeed, several studies report how a prolonged time of residence within an EMP state either slows or completely blocks the reversal to an epithelial phenotype upon withdrawal of stimuli[19,114,116,231,232]. Such phenomena can be explained by a two-tier mechanism in which an acute transcriptional response is followed by slower but more persistent epigenetic reprogramming (Fig. 3f). Indeed, during EMT, the generation of epigenetic marks can lag behind transcriptional changes, both in the case of both DNA methylation[229] and histone modification[232]. Progressive generation of these epigenetic marks may then cause a gradual silencing of alternative states. Alternatively, epigenetic marks can regulate the thresholds for transcription factors to alter expression of their downstream targets[231]. In each case, epigenetic marks provide an EMT memory that stabilizes the current state[114,229,231,232].

Ribosomal and translational regulation of EMP

Several pathologies link ribosomal functioning to type 1 and type 2 EMP[233]. Neurocristopathies, that is, disorders of neural crest delamination during embryogenesis, can result from mutations in canonical EMT-TFs[89-92]. However, other neurocristopathies result from ribosomal defects. For example, Diamond–Blackfan anaemia is linked to mutations in at least 14 ribosomal proteins (RPs)[234-236], and Treacher–Collins syndrome is associated with defects in RNA polymerase I (RNA Pol I)[237], or TCOF1[238], which are both involved in rRNA transcription, as well as defects in RNA Pol III[237], which is required for 5S rRNA and tRNA transcription. Similarly, several pathologies link type 2 EMT with translation. Sera of patients with systemic scleroderma, a disease characterized by fibrosis of the lungs and skin, frequently contain autoantibodies against RNA Pol III[239] or fibrillarin[240,241], a factor involved in the pre-processing of rRNA[242]. Moreover, other fibrosis-manifesting pathologies, such as

dyskeratosis congenita[243], childhood cirrhosis[244,245] and atrial fibrillation[246], are linked to defects in ribosome formation.

Recently, elevated ribosome biogenesis was identified as a general feature during both *in vivo* and *in vitro* EMT[247,248]. This association is maintained across distinct EMT triggers and species. Intriguingly, although ribosomal biogenesis is generally cell cycle-dependent (being linked with cell proliferation and growth), EMT-associated ribosomal biogenesis coincided with a G1/S arrest. Enhanced rRNA synthesis during EMT is linked with chromatin remodelling and recruitment of RNA Pol I and SNAI1 to rDNA operons[247]. Interestingly, incorporation of external eukaryotic or prokaryotic ribosomes in somatic cells causes an upregulation of EMT-TFs[249,250]. Furthermore, mRNAs of RPs can show strong enrichment at the migrating edge of mesenchymal-like cells[251-253]. EMT causes an upregulation of the RNA binding protein LARP6, which recruits RP-mRNAs specifically to cellular protrusions, transforming these protrusions into hotspots for RP-mRNA translation and, consequently, increasing overall protein synthesis[254].

Additionally, several post-transcriptional mechanisms regulate EMP at a translational level during cancer progression[233]. N^6-methyladenosine (**m6A**) methylation of *SNAI1* mRNA by METTL3 enhances cap-independent translation of *SNAI1*[253,255]. Moreover, the ribosome-binding protein CELF1 attaches to the 3' untranslated region of the *SNAI1* mRNA, further enhancing translation[256]. Furthermore, YB-1, which is aberrantly expressed across cancers, promotes internal ribosome entry site (IRES)-mediated translation initiation of ZEB2, SNAI1 and TWIST[257]. Additionally, mTOR signalling, a regulator of cap-dependent translation, inactivates 4E-BP1, an inhibitor of the elongation factor eIF4E, resulting in elevated expression of SNAI1, TWIST and vimentin in colorectal cancer cells[258,259]. Lastly, targeting translation, for example, with RNA Pol I inhibitors[247] or by blocking ribosome export from the nucleus[260], has been shown to inhibit EMT. Together these results underline the regulation of EMP at the translational level, and the possibility for therapeutic intervention at this level (Fig. 5g).

Metabolic rewiring during EMP underlies functional changes

EMP demands thorough rewiring of cellular metabolic networks[261-265] (Fig. 5h). Importantly, although we distil general principles emerging across studies, the highly

complex nature of metabolic networks cautions against extrapolation from one context to another.

Dysregulated lipid metabolism modulates EMP[261-264]. Cancers display increased lipogenesis[266-268], for example, through upregulation of fatty acid synthase (FAS)[266-268]. In turn, upregulation or inhibition of FAS promotes or supresses EMT, respectively[269-276]. Additionally, the lipid composition of the cell membrane dictates its fluidity. Membrane fluidity in turn determines cell motility[277-281]. As such, phosphatidylcholine[282] and sphingosine-1-phosphate[283-291], which liquify the membrane[292,293], promote EMT. Conversely, ceramides[278,288,294] and cholesterol[280,281,295], which rigidify the membrane[296,297], negatively affect EMT. Following this trend, fatty acids in mesenchymal-like cells are on average shorter[282], and more unsaturated[282,298]. Lastly, aberrant synthesis of lipid signalling molecules can activate downstream signalling cascades; for example, the PPAR transcription factor family, activated by fatty acid ligands, inhibits EMT both in cancer cells[299-302] and in fibrosis[303-307]. Furthermore, eicosanoids regulate EMP; whereas prostaglandin E2 promotes EMT[308-312], lipoxin A4 inhibits type 1[313], type 2[314-316] and type 3[317,318] EMT.

In cancer, the shift from oxidative phosphorylation (OxPhos) to aerobic glycolysis[319] induces EMT through various mechanisms[261-264]. Firstly, increased glycolytic flux requires upregulation of key glycolytic enzymes and glucose transporters, both of which can induce EMT[320-324]. Importantly, metabolic enzymes can also drive EMT through non-metabolic means; for example, pyruvate kinase M2 drives EMT-associated gene transcription after translocation to the nucleus where it interacts with various transcription factors[320,321]. Secondly, cancers often display mitochondrial dysfunction, impairing OxPhos and further enhancing EMT[325-328]. Thirdly, secretion of acidic, glycolytic products, such as lactic acid results in acidification of the microenvironment, further promoting EMT[329-333]. In this manner, EMT in cancer once again echoes early development: in the neural crest[334] and the presomatic mesoderm[335,336], glycolysis and OxPhos are associated with EMT and MET, respectively[337].

The parallels between cancer-associated and EMP-associated metabolic rewiring described above — that is, increased glycolysis and lipogenesis, and reduced OxPhos — suggest that cancer cell metabolism favours EMP. Even so, different EMP states display distinct metabolic activity[338-340]. For example, catalytic activity of

phosphoglycerate dehydrogenase (PHGDH) supports cancer cell proliferation in triple-negative breast cancer. However, loss of PHGDH re-routes glycolytic intermediates towards the hexosamine-sialic acid pathway, resulting in sialylation of $\alpha_v\beta_3$ integrin, which drives EMT and metastasis[338]. Highly migratory, mesenchymal-like breast cancer cells showed increased glycolysis compared with slower, more epithelial-like cells with increased OxPhos[339]. Similarly, whereas enhanced nucleotide synthesis is crucial for cancer cell proliferation, pyrimidine catabolism seems to support the mesenchymal state[340]. As cells undergoing EMT redeem proliferative capacity for migratory ability, the associated metabolic rewiring may be understood as the re-routing of resources from growth to motility.

The interplay between EMP and metabolism provides therapeutic opportunities. As distinct EMP states show distinct nutrient dependencies, depletion of certain nutrients might enable targeting of mesenchymal-like cells. Alternatively, pharmacological disruption of key nodes in the metabolic network may supress EMP and, thus, limit tumour progression[262,264,341].

Phenotypic stability factors stabilize hybrid EMT states

Whereas complete EMT is a rare event in cancer, partial EMT is much more prevalent[4,342]. The resulting hybrid E/M state possesses properties distinct from cells at either end of the EMT spectrum. First, hybrid states show enhanced stemness, as has been thoroughly reviewed[343,344]. In cancer, hybrid E/M cells are enriched in circulating tumour cells (CTCs)[345,346] and display enhanced metastatic potential[18,22,23,342,347]. Additionally, CTCs must weather a variety of environments before seeding at secondary tumour sites. During this journey, the hybrid state may provide a survival advantage through its intrinsic adaptability[348] or through the formation of CTC clusters[50,349,350].

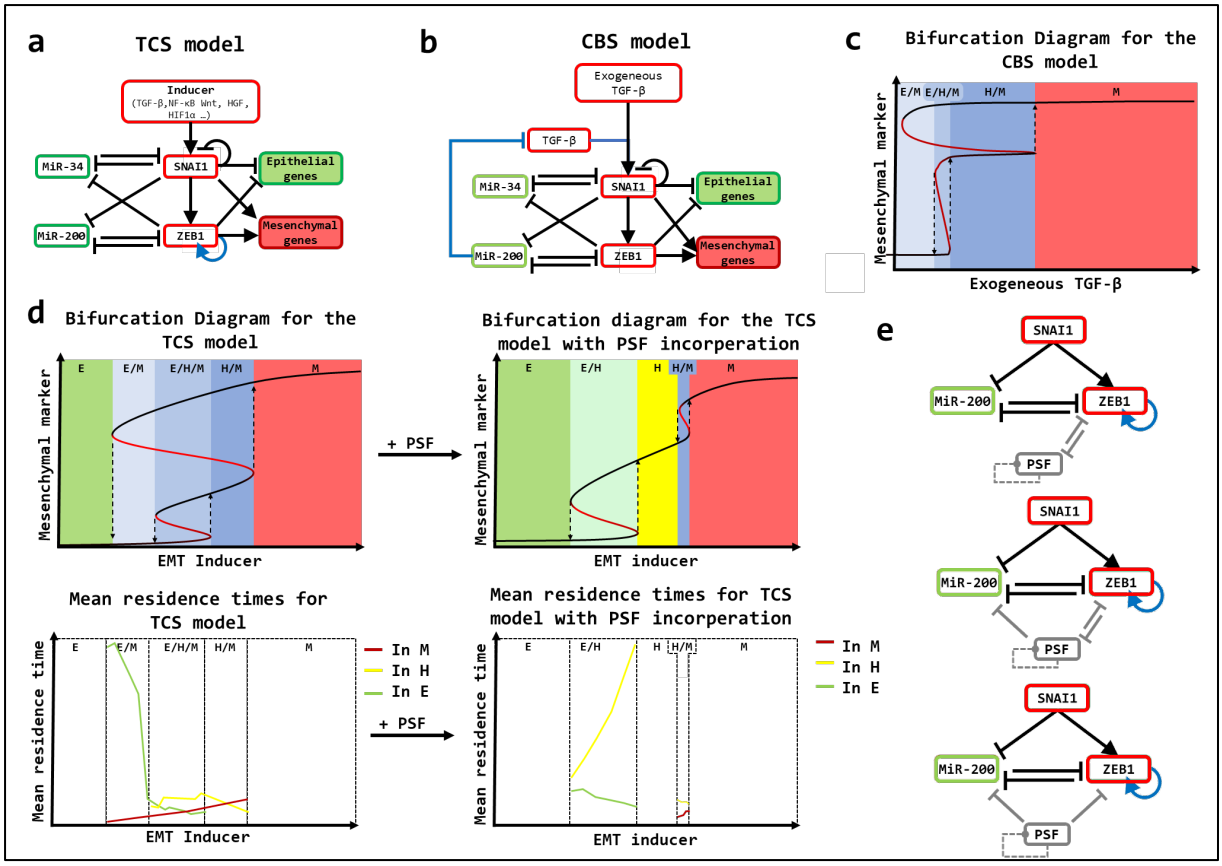


Fig. 6: Phenotypic stability factors interact with the core regulatory EMT network to stabilize the hybrid EMT state

Regulators of epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) processes converge on a core regulatory circuit consisting of the ZEB–miR-200 and the SNAI1–miR-34 double-negative feedback loops. Epithelial states (E) display high miR-200 and miR-34 expression, whereas mesenchymal states (M) show increased EMT-associated transcription factor (EMT-TF) expression. Phenotypic stability factors (PSFs) act as molecular brakes by impeding complete transition to a mesenchymal state and stabilizing hybrid (H) epithelial and mesenchymal states. **a, b|** The ternary chimaera switch (TCS) model[198], which includes self-activation of ZEB1 (panel **a**), and the cascading bistable switches (CBS) model[197], which does not (panel **b**). Factors that promote the epithelial or mesenchymal phenotype are marked in green or red, respectively. Differences between the models are marked in blue. Both models predict a tri-stable system consisting of an epithelial, mesenchymal, and hybrid EMT state. Part a adapted with permission from ref. 184, PNAS. Part b adapted with permission from ref. 185, AAAS. **c, d|** Bifurcation diagrams to plot cell state transitions in function of an EMT-inducing signal. Importantly, these bifurcation diagrams are the result of deterministic models, not incorporating stochastic events. **c|** Bifurcation diagram of the CBS model[198]. Black lines display stable states, red lines represent unstable states. Dotted lines with arrows represent transitions between states. The diagram shows hysteretic features as there is an asymmetry between transitions during increasing versus decreasing TGF-β. In the CBS model, the mesenchymal state, once reached, may be maintained indefinitely (as is shown here) depending on the strength of the endogenous TGF-β production. Part c adapted with permission from ref. 185, AAAS. **d|** Bifurcation diagrams and mean residence times for the TCS model in the absence and presence of a phenotypic stability factor (PSF)[351] (panel **d**): incorporation of a PSF in increases the range of parameters in which the hybrid EMT state exists. Additionally, in the presence of a PSF, a range of parameters exists which allows for the presence of a monostable, hybrid EMT population. Lastly, using stochastic models to simulate cell state transitions, PSFs increase the time that cells spend, on average, in the hybrid state. We believe the networks and diagrams presented are of value as they illustrate the general mechanisms of the PSFs. Part d adapted with permission from ref. 351, IOP. **e|** Simplified circuit focussing on the *miR-200*-ZEB inhibitory loop with incorporation of common network motifs which can stabilize the hybrid EMT phenotype[352] (in grey). The dotted feedback loop ending in a circle represents that these factors may display autoregulatory behaviour, either self-activating or self-inhibiting behaviour. These network

motifs can be used to identify additional PSFs. Notably, factors which form a double-negative feedback loop with *MiR-200* instead of ZEB1 do not stabilize the hybrid EMT phenotype. Similarly, whereas factors inhibiting both ZEB1 and *miR-200* promote a hybrid phenotype, factors activating both ZEB1 and *miR-200* are unable to do so. Part e is adapted from ref. 343, CC BY 4.0.

Several mediators, termed phenotypic stability factors (PSFs) have been shown to stabilize hybrid E/M states (Fig. 6d-e). These PSFs can be thought of as molecular brakes, delaying the progression from a hybrid E/M state to a fully mesenchymal state. Examples include the OVOL transcription factor family[352,353], NUMB[354], NUMBL[354], NFATC1[355], NRF2[356,357] (also known as NFE2L2), GRHL2[352,358], the p63 protein isoform ΔNP63α[222,359], *miR-145*/OCT4[352] and the classical EMT-TF SNAI2[360]. PSFs generally modulate the EMP landscape in three major ways (Fig. 6d). First, the presence of each PSF individually has been shown to increase the range of parameters in which hybrid E/M states exist. Second, considering solely the core regulatory network, populations of hybrid cells will always co-exist with fully epithelial or mesenchymal states. Inclusion of several PSFs independently in the EMP-governing regulatory network allows for the presence of monostable hybrid populations in certain conditions. Last, in environments enabling coexisting populations of hybrid states with epithelial and/or mesenchymal states, several individual PSFs have been shown to increase the time that individual cells spend, on average, in the hybrid state[351]. Common network motifs to identify additional PSFs were proposed[352] (Fig. 6e).

Targeting factors that stabilize hybrid E/M states could dissolve CTC clusters and reduce stemness and plasticity of CTCs, consequently preventing metastatic outgrowth. Knockdown of a single of the above-mentioned PSF is sufficient to cause a shift towards full EMT. For example, independent knockdown of GRHL2[352], OVOL2[352], NUMB[354], NUMBL[354], NFATC1[355], or NRF2[357] in H1975 non-small cell lung cancer cells caused a shift from a hybrid E/M state to a mesenchymal state. As such, the non-redundant character of these factors may prove them good candidates for therapeutic strategies seeking to disrupt the partial EMT phenotype and prevent metastasis.

EMT-TFs beyond epithelial plasticity

The role of the so-called EMT-TFs is not restricted to epithelial cells. These transcription factors are abundantly expressed, and play essential roles in, several other lineages including neurons[361-363], myocytes[364,365], adipocytes[366], haematopoietic cells[367-369] and melanocytes[370,371]. Strikingly, the functions of downstream targets identified in these lineages are largely overlapping with those observed during classical EMT; genes involved in cell adhesion, migration, chemotaxis and growth factor receptors. An additional parallel in these lineages is that aberrant upregulation of EMT-TFs is sufficient to drive oncogenic transformation, and is associated with increased stem cell properties and resistance to therapy[275,372-375].

Whereas EMT-TFs work in concert during classical EMT, loss or gain of different EMT-TFs in other lineages can result in different, sometimes opposing phenotypes. As such, EMT-TFs demonstrate overlapping but distinct functions depending on the intracellular and microenvironmental context[372,376-378]. This pleiotropism presents an opportunity: the plasticity of EMP-displaying cancer cells could be exploited to force transdifferentiation towards non-malignant, post-mitotic cell types. Indeed, the induction of adipocyte differentiation in EMP-displaying breast cancer cells was shown to significantly repress invasion and metastasis *in vivo*[379]. This example highlights how a better understanding of the hierarchy of and coordination between EMT-TFs may teach us how to manipulate the EMP landscape for therapeutic purposes. Additionally, this precedent begs the question of how we distinguish EMP from other types of cellular plasticity.

Conclusions and future perspectives

In recent years, a surge in the use of single-cell profiling methods has driven new insights in the EMP field. Such methods have enabled the identification and characterization of distinct transition states across the EMP spectrum[9,11-21]. Additionally, time-course analysis has made clear that EMT and MET are distinct, asymmetric processes[9,19,115]. The functional characteristics associated with MET states, or the ‘EMP memory’ retained after concurrent EMT and MET transitions, may be vital in understanding and tackling metastasis.

To tackle the ever-increasing complexity of gene regulatory networks mediating EMP, some researchers have turned to systems biology approaches. These

approaches allow large-scale modelling and simulated perturbations of gene regulatory networks, driving the generation of new hypotheses. Additionally, mathematical modelling allows the study of phenomena which are difficult to investigate experimentally, for example, the dynamics of cell state transitions[127-129,157,197,198,231], the stabilization of partial EMT states[222,352-360], or the effects of noise on cellular plasticity[128,129,134,136,138,139].

Key questions remain regarding the link between EMP and malignancy. Is a certain EMP state required for therapy resistance or metastasis? Is it the plasticity itself or the population-level heterogeneity that confers these EMP-associated characteristics? Which of the many states or transitions should we target therapeutically? Additionally, new questions emerge from novel insights. For example, to what extent does cellular noise drive pathological EMP? And, might this noise be targeted therapeutically? Importantly, whereas dozens of individual EMP programmes have been analysed in depth, the search for unifying principles, consistent across systems, continues.

References

1 Waddington, C. H. *The strategy of the genes*. (Routledge, 2014).

2 Kalluri, R. & Weinberg, R. A. The basics of epithelial-mesenchymal transition. *J Clin Invest* **119**, 1420-1428, doi:10.1172/JCI39104 (2009).

3 Nieto, M. A., Huang, R. Y., Jackson, R. A. & Thiery, J. P. Emt: 2016. *Cell* **166**, 21-45, doi:10.1016/j.cell.2016.06.028 (2016).

4 Yang, J. *et al.* Guidelines and definitions for research on epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* **21**, 341-352, doi:10.1038/s41580-020-0237-9 (2020).

5 Hay, E. D. in *Epithelial-Mesenchymal Interactions; 18th Hahnemann Symposium, 1968.* (Williams & Wilkins).

6 Hay, E. D. An overview of epithelio-mesenchymal transformation. *Acta Anat (Basel)* **154**, 8-20, doi:10.1159/000147748 (1995).

7 Thiery, J. P. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* **2**, 442-454, doi:10.1038/nrc822 (2002).

8 Lamouille, S., Xu, J. & Derynck, R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* **15**, 178-196, doi:10.1038/nrm3758 (2014).

9 Cook, D. P. & Vanderhyden, B. C. Context specificity of the EMT transcriptional response. *Nat Commun* **11**, 2142, doi:10.1038/s41467-020-16066-2 (2020).

10 Jolly, M. K., Ware, K. E., Gilja, S., Somarelli, J. A. & Levine, H. EMT and MET: necessary or permissive for metastasis? *Mol Oncol* **11**, 755-769, doi:10.1002/1878-0261.12083 (2017).

11 Dong, J. *et al.* Single-cell RNA-seq analysis unveils a prevalent epithelial/mesenchymal hybrid state during mouse organogenesis. *Genome Biol* **19**, 31, doi:10.1186/s13059-018-1416-2 (2018).

12 Puram, S. V. *et al.* Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer. *Cell* **171**, 1611-1624 e1624, doi:10.1016/j.cell.2017.10.044 (2017).

13 Zhao, J. *et al.* Single cell RNA-seq reveals the landscape of tumor and infiltrating immune cells in nasopharyngeal carcinoma. *Cancer Lett* **477**, 131-143, doi:10.1016/j.canlet.2020.02.010 (2020).

14 Ji, A. L. *et al.* Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma. *Cell* **182**, 1661-1662, doi:10.1016/j.cell.2020.08.043 (2020).

15 McFaline-Figueroa, J. L. *et al.* A pooled single-cell genetic screen identifies regulatory checkpoints in the continuum of the epithelial-to-mesenchymal transition. *Nat Genet* **51**, 1389-1398, doi:10.1038/s41588-019-0489-5 (2019).

16 Deshmukh, A. P. *et al.* Identification of EMT signaling cross-talk and gene regulatory networks by single-cell RNA sequencing. *Proc Natl Acad Sci U S A* **118**, doi:10.1073/pnas.2102050118 (2021).

17 Simeonov, K. P. *et al.* Single-cell lineage tracing of metastatic cancer reveals selection of hybrid EMT states. *Cancer Cell* **39**, 1150-1162 e1159, doi:10.1016/j.ccell.2021.05.005 (2021).

18 Pastushenko, I. *et al.* Identification of the tumour transition states occurring during EMT. *Nature* **556**, 463-468, doi:10.1038/s41586-018-0040-3 (2018).

19 Karacosta, L. G. *et al.* Mapping lung cancer epithelial-mesenchymal transition states and trajectories with single-cell resolution. *Nat Commun* **10**, 5587, doi:10.1038/s41467-019-13441-6 (2019).

20 Taverna, J. A. *et al.* Single-Cell Proteomic Profiling Identifies Combined AXL and JAK1 Inhibition as a Novel Therapeutic Strategy for Lung Cancer. *Cancer Res* **80**, 1551-1563, doi:10.1158/0008-5472.CAN-19-3183 (2020).

21 Pastushenko, I. & Blanpain, C. EMT Transition States during Tumor Progression and Metastasis. *Trends Cell Biol* **29**, 212-226, doi:10.1016/j.tcb.2018.12.001 (2019).

22 Pastushenko, I. *et al.* Fat1 deletion promotes hybrid EMT state, tumour stemness and metastasis. *Nature* **589**, 448-455, doi:10.1038/s41586-020-03046-1 (2021).

23 Kroger, C. *et al.* Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells. *Proc Natl Acad Sci U S A* **116**, 7353-7362, doi:10.1073/pnas.1812876116 (2019).
24 Potts, J. D. & Runyan, R. B. Epithelial-mesenchymal cell transformation in the embryonic heart can be mediated, in part, by transforming growth factor beta. *Dev Biol* **134**, 392-401, doi:10.1016/0012-1606(89)90111-5 (1989).
25 Miettinen, P. J., Ebner, R., Lopez, A. R. & Derynck, R. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. *J Cell Biol* **127**, 2021-2036, doi:10.1083/jcb.127.6.2021 (1994).
26 Stoker, M. & Perryman, M. An epithelial scatter factor released by embryo fibroblasts. *J Cell Sci* **77**, 209-223, doi:10.1242/jcs.77.1.209 (1985).
27 Valles, A. M. *et al.* Acidic fibroblast growth factor is a modulator of epithelial plasticity in a rat bladder carcinoma cell line. *Proc Natl Acad Sci U S A* **87**, 1124-1128, doi:10.1073/pnas.87.3.1124 (1990).
28 Kim, K., Lu, Z. & Hay, E. D. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. *Cell Biol Int* **26**, 463-476, doi:10.1006/cbir.2002.0901 (2002).
29 Timmerman, L. A. *et al.* Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. *Genes Dev* **18**, 99-115, doi:10.1101/gad.276304 (2004).
30 Lester, R. D., Jo, M., Montel, V., Takimoto, S. & Gonias, S. L. uPAR induces epithelial-mesenchymal transition in hypoxic breast cancer cells. *J Cell Biol* **178**, 425-436, doi:10.1083/jcb.200701092 (2007).
31 Yang, M. H. *et al.* Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat Cell Biol* **10**, 295-305, doi:10.1038/ncb1691 (2008).
32 Loayza-Puch, F. *et al.* TGFbeta1-induced leucine limitation uncovered by differential ribosome codon reading. *EMBO Rep* **18**, 549-557, doi:10.15252/embr.201744000 (2017).
33 Recouvreux, M. V. *et al.* Glutamine depletion regulates Slug to promote EMT and metastasis in pancreatic cancer. *J Exp Med* **217**, doi:10.1084/jem.20200388 (2020).
34 Nakasuka, F. *et al.* TGF-beta-dependent reprogramming of amino acid metabolism induces epithelial-mesenchymal transition in non-small cell lung cancers. *Commun Biol* **4**, 782, doi:10.1038/s42003-021-02323-7 (2021).
35 Heise, R. L., Stober, V., Cheluvaraju, C., Hollingsworth, J. W. & Garantziotis, S. Mechanical stretch induces epithelial-mesenchymal transition in alveolar epithelia via hyaluronan activation of innate immunity. *J Biol Chem* **286**, 17435-17444, doi:10.1074/jbc.M110.137273 (2011).
36 Przybyla, L., Muncie, J. M. & Weaver, V. M. Mechanical Control of Epithelial-to-Mesenchymal Transitions in Development and Cancer. *Annu Rev Cell Dev Biol* **32**, 527-554, doi:10.1146/annurev-cellbio-111315-125150 (2016).
37 Zhao, X., Hu, J., Li, Y. & Guo, M. Volumetric compression develops noise-driven single-cell heterogeneity. *Proc Natl Acad Sci U S A* **118**, doi:10.1073/pnas.2110550118 (2021).
38 Fattet, L. *et al.* Matrix Rigidity Controls Epithelial-Mesenchymal Plasticity and Tumor Metastasis via a Mechanoresponsive EPHA2/LYN Complex. *Dev Cell* **54**, 302-316 e307, doi:10.1016/j.devcel.2020.05.031 (2020).
39 Deng, Y., Chakraborty, P., Jolly, M. K. & Levine, H. A Theoretical Approach to Coupling the Epithelial-Mesenchymal Transition (EMT) to Extracellular Matrix (ECM) Stiffness via LOXL2. *Cancers (Basel)* **13**, doi:10.3390/cancers13071609 (2021).
40 Gao, Y. *et al.* Metastasis Organotropism: Redefining the Congenial Soil. *Dev Cell* **49**, 375-391, doi:10.1016/j.devcel.2019.04.012 (2019).
41 Labelle, M., Begum, S. & Hynes, R. O. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell* **20**, 576-590, doi:10.1016/j.ccr.2011.09.009 (2011).

42 Kan, T. *et al.* Single-cell EMT-related transcriptional analysis revealed intra-cluster heterogeneity of tumor cell clusters in epithelial ovarian cancer ascites. *Oncogene* **39**, 4227-4240, doi:10.1038/s41388-020-1288-2 (2020).
43 Szczerba, B. M. *et al.* Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* **566**, 553-+, doi:10.1038/s41586-019-0915-y (2019).
44 Liu, Q., Liao, Q. & Zhao, Y. Myeloid-derived suppressor cells (MDSC) facilitate distant metastasis of malignancies by shielding circulating tumor cells (CTC) from immune surveillance. *Med Hypotheses* **87**, 34-39, doi:10.1016/j.mehy.2015.12.007 (2016).
45 Sprouse, M. L. *et al.* PMN-MDSCs Enhance CTC Metastatic Properties through Reciprocal Interactions via ROS/Notch/Nodal Signaling. *Int J Mol Sci* **20**, doi:10.3390/ijms20081916 (2019).
46 Hurtado, P., Martinez-Pena, I. & Pineiro, R. Dangerous Liaisons: Circulating Tumor Cells (CTCs) and Cancer-Associated Fibroblasts (CAFs). *Cancers (Basel)* **12**, doi:10.3390/cancers12102861 (2020).
47 Xiong, G. *et al.* Hsp47 promotes cancer metastasis by enhancing collagen-dependent cancer cell-platelet interaction. *Proc Natl Acad Sci U S A* **117**, 3748-3758, doi:10.1073/pnas.1911951117 (2020).
48 Hou, J. M. *et al.* Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. *J Clin Oncol* **30**, 525-532, doi:10.1200/JCO.2010.33.3716 (2012).
49 Paoli, P., Giannoni, E. & Chiarugi, P. Anoikis molecular pathways and its role in cancer progression. *Biochim Biophys Acta* **1833**, 3481-3498, doi:10.1016/j.bbamcr.2013.06.026 (2013).
50 Giuliano, M. *et al.* Perspective on Circulating Tumor Cell Clusters: Why It Takes a Village to Metastasize. *Cancer Res* **78**, 845-852, doi:10.1158/0008-5472.CAN-17-2748 (2018).
51 Aceto, N. *et al.* Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158**, 1110-1122, doi:10.1016/j.cell.2014.07.013 (2014).
52 Cheung, K. J. *et al.* Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. *Proc Natl Acad Sci U S A* **113**, E854-863, doi:10.1073/pnas.1508541113 (2016).
53 Sarioglu, A. F. *et al.* A microfluidic device for label-free, physical capture of circulating tumor cell clusters. *Nat Methods* **12**, 685-691, doi:10.1038/nmeth.3404 (2015).
54 Beard, J. Embryological aspects and etiology of carcinoma. *The Lancet* **159**, 1758-1761 (1902).
55 Virchow, R. Vorlesungen über Cellularpathologie in ihrer Begründung auf physiologischer und pathologischer Gewebelehre. *Berlin: Verlag August Hirschwald* (1858).
56 Markert, C. L. Neoplasia: a disease of cell differentiation. *Cancer Res* **28**, 1908-1914 (1968).
57 Micalizzi, D. S., Farabaugh, S. M. & Ford, H. L. Epithelial-Mesenchymal Transition in Cancer: Parallels Between Normal Development and Tumor Progression. *J Mammary Gland Biol* **15**, 117-134, doi:10.1007/s10911-010-9178-9 (2010).
58 Nieto, M. A. Epithelial plasticity: a common theme in embryonic and cancer cells. *Science* **342**, 1234850, doi:10.1126/science.1234850 (2013).
59 Thiery, J. P., Acloque, H., Huang, R. Y. & Nieto, M. A. Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871-890, doi:10.1016/j.cell.2009.11.007 (2009).
60 Pritchard, C. *et al.* Conserved gene expression programs integrate mammalian prostate development and tumorigenesis. *Cancer Res* **69**, 1739-1747, doi:10.1158/0008-5472.CAN-07-6817 (2009).
61 Pomerantz, M. M. *et al.* Prostate cancer reactivates developmental epigenomic programs during metastatic progression. *Nat Genet* **52**, 790-799, doi:10.1038/s41588-020-0664-8 (2020).

62 Schaeffer, E. M. *et al.* Androgen-induced programs for prostate epithelial growth and invasion arise in embryogenesis and are reactivated in cancer. *Oncogene* **27**, 7180-7191, doi:10.1038/onc.2008.327 (2008).
63 Roe, J. S. *et al.* Enhancer Reprogramming Promotes Pancreatic Cancer Metastasis. *Cell* **170**, 875-888 e820, doi:10.1016/j.cell.2017.07.007 (2017).
64 Gupta, P. B. *et al.* The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. *Nat Genet* **37**, 1047-1054, doi:10.1038/ng1634 (2005).
65 Holczbauer, A. *et al.* Modeling pathogenesis of primary liver cancer in lineage-specific mouse cell types. *Gastroenterology* **145**, 221-231, doi:10.1053/j.gastro.2013.03.013 (2013).
66 Latil, M. *et al.* Cell-Type-Specific Chromatin States Differentially Prime Squamous Cell Carcinoma Tumor-Initiating Cells for Epithelial to Mesenchymal Transition. *Cell Stem Cell* **20**, 191-204 e195, doi:10.1016/j.stem.2016.10.018 (2017).
67 Chou, J., Provot, S. & Werb, Z. GATA3 in development and cancer differentiation: cells GATA have it! *Journal of cellular physiology* **222**, 42-49 (2010).
68 Bai, F. *et al.* GATA3 functions downstream of BRCA1 to suppress EMT in breast cancer. *Theranostics* **11**, 8218 (2021).
69 Saotome, M., Poduval, D. B., Nair, R., Cooper, M. & Takaku, M. GATA3 truncation mutants alter EMT related gene expression via partial motif recognition in luminal breast cancer cells. *Frontiers in Genetics* **13**, 67 (2022).
70 Kouros-Mehr, H. *et al.* GATA-3 links tumor differentiation and dissemination in a luminal breast cancer model. *Cancer cell* **13**, 141-152 (2008).
71 Yan, W., Cao, Q. J., Arenas, R. B., Bentley, B. & Shao, R. GATA3 inhibits breast cancer metastasis through the reversal of epithelial-mesenchymal transition. *Journal of Biological Chemistry* **285**, 14042-14051 (2010).
72 Li, Y. *et al.* Loss of GATA3 in bladder cancer promotes cell migration and invasion. *Cancer Biology & Therapy* **15**, 428-435 (2014).
73 Chakrabarti, R. *et al.* Elf5 inhibits the epithelial–mesenchymal transition in mammary gland development and breast cancer metastasis by transcriptionally repressing Snail2. *Nature cell biology* **14**, 1212-1222 (2012).
74 Wu, B. *et al.* Epigenetic regulation of Elf5 is associated with epithelial-mesenchymal transition in urothelial cancer. *PloS one* **10**, e0117510 (2015).
75 Yao, B. *et al.* Elf5 inhibits TGF-β-driven epithelial-mesenchymal transition in prostate cancer by repressing SMAD3 activation. *The Prostate* **75**, 872-882 (2015).
76 Song, Y., Washington, M. K. & Crawford, H. C. Loss of FOXA1/2 is essential for the epithelial-to-mesenchymal transition in pancreatic cancer. *Cancer Res* **70**, 2115-2125, doi:10.1158/0008-5472.CAN-09-2979 (2010).
77 Teng, M., Zhou, S., Cai, C., Lupien, M. & He, H. H. Pioneer of prostate cancer: past, present and the future of FOXA1. *Protein Cell* **12**, 29-38, doi:10.1007/s13238-020-00786-8 (2021).
78 Tiwari, N. *et al.* Klf4 is a transcriptional regulator of genes critical for EMT, including Jnk1 (Mapk8). *PLoS One* **8**, e57329, doi:10.1371/journal.pone.0057329 (2013).
79 Agbo, K. C. *et al.* Loss of the Kruppel-like factor 4 tumor suppressor is associated with epithelial-mesenchymal transition in colorectal cancer. *J Cancer Metastasis Treat* **5**, doi:10.20517/2394-4722.2019.35 (2019).
80 Zeisberg, E. M., Potenta, S., Xie, L., Zeisberg, M. & Kalluri, R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. *Cancer Res* **67**, 10123-10128, doi:10.1158/0008-5472.CAN-07-3127 (2007).
81 Pardali, E., Sanchez-Duffhues, G., Gomez-Puerto, M. C. & Ten Dijke, P. TGF-beta-Induced Endothelial-Mesenchymal Transition in Fibrotic Diseases. *Int J Mol Sci* **18**, doi:10.3390/ijms18102157 (2017).

82 Zeisberg, E. M., Potenta, S. E., Sugimoto, H., Zeisberg, M. & Kalluri, R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol* **19**, 2282-2287, doi:10.1681/ASN.2008050513 (2008).
83 Zeisberg, E. M. *et al.* Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* **13**, 952-961, doi:10.1038/nm1613 (2007).
84 Tombor, L. S. *et al.* Single cell sequencing reveals endothelial plasticity with transient mesenchymal activation after myocardial infarction. *Nat Commun* **12**, 681, doi:10.1038/s41467-021-20905-1 (2021).
85 Lin, S. C. *et al.* Endothelial-to-Osteoblast Conversion Generates Osteoblastic Metastasis of Prostate Cancer. *Dev Cell* **41**, 467-480 e463, doi:10.1016/j.devcel.2017.05.005 (2017).
86 Medici, D. *et al.* Conversion of vascular endothelial cells into multipotent stem-like cells. *Nat Med* **16**, 1400-1406, doi:10.1038/nm.2252 (2010).
87 De Craene, B. & Berx, G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer* **13**, 97-110, doi:10.1038/nrc3447 (2013).
88 Acloque, H., Adams, M. S., Fishwick, K., Bronner-Fraser, M. & Nieto, M. A. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. *J Clin Invest* **119**, 1438-1449, doi:10.1172/JCI38019 (2009).
89 Sanchez-Martin, M. *et al.* SLUG (SNAI2) deletions in patients with Waardenburg disease. *Hum Mol Genet* **11**, 3231-3236, doi:10.1093/hmg/11.25.3231 (2002).
90 Zweier, C. *et al.* "Mowat-Wilson" syndrome with and without Hirschsprung disease is a distinct, recognizable multiple congenital anomalies-mental retardation syndrome caused by mutations in the zinc finger homeo box 1B gene. *Am J Med Genet* **108**, 177-181 (2002).
91 el Ghouzzi, V. *et al.* Mutations of the TWIST gene in the Saethre-Chotzen syndrome. *Nat Genet* **15**, 42-46, doi:10.1038/ng0197-42 (1997).
92 Howard, T. D. *et al.* Mutations in TWIST, a basic helix-loop-helix transcription factor, in Saethre-Chotzen syndrome. *Nat Genet* **15**, 36-41, doi:10.1038/ng0197-36 (1997).
93 Horiguchi, K. *et al.* Role of Ras signaling in the induction of snail by transforming growth factor-beta. *J Biol Chem* **284**, 245-253, doi:10.1074/jbc.M804777200 (2009).
94 Wang, Y. *et al.* Critical role for transcriptional repressor Snail2 in transformation by oncogenic RAS in colorectal carcinoma cells. *Oncogene* **29**, 4658-4670, doi:10.1038/onc.2010.218 (2010).
95 Zhang, X., Cheng, Q., Yin, H. & Yang, G. Regulation of autophagy and EMT by the interplay between p53 and RAS during cancer progression (Review). *Int J Oncol* **51**, 18-24, doi:10.3892/ijo.2017.4025 (2017).
96 Pylayeva-Gupta, Y., Grabocka, E. & Bar-Sagi, D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer* **11**, 761-774, doi:10.1038/nrc3106 (2011).
97 Bisogno, L. S., Friedersdorf, M. B. & Keene, J. D. Ras Post-transcriptionally Enhances a Pre-malignantly Primed EMT to Promote Invasion. *iScience* **4**, 97-108, doi:10.1016/j.isci.2018.05.011 (2018).
98 Yoh, K. E. *et al.* Repression of p63 and induction of EMT by mutant Ras in mammary epithelial cells. *Proc Natl Acad Sci U S A* **113**, E6107-E6116, doi:10.1073/pnas.1613417113 (2016).
99 Grassian, A. R. *et al.* Isocitrate dehydrogenase (IDH) mutations promote a reversible ZEB1/microRNA (miR)-200-dependent epithelial-mesenchymal transition (EMT). *J Biol Chem* **287**, 42180-42194, doi:10.1074/jbc.M112.417832 (2012).
100 Lu, J. *et al.* IDH1 mutation promotes proliferation and migration of glioma cells via EMT induction. *J BUON* **24**, 2458-2464 (2019).
101 Jiang, Z. *et al.* RB1 and p53 at the crossroad of EMT and triple-negative breast cancer. *Cell Cycle* **10**, 1563-1570, doi:10.4161/cc.10.10.15703 (2011).
102 Fedele, V. & Melisi, D. Permissive State of EMT: The Role of Immune Cell Compartment. *Front Oncol* **10**, 587, doi:10.3389/fonc.2020.00587 (2020).

103 Quaresma, M. C. *et al.* Mutant CFTR Drives TWIST1 mediated epithelial-mesenchymal transition. *Cell Death Dis* **11**, 920, doi:10.1038/s41419-020-03119-z (2020).
104 Gibson, R. L., Burns, J. L. & Ramsey, B. W. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* **168**, 918-951, doi:10.1164/rccm.200304-505SO (2003).
105 Nichols, D., Chmiel, J. & Berger, M. Chronic inflammation in the cystic fibrosis lung: alterations in inter- and intracellular signaling. *Clin Rev Allergy Immunol* **34**, 146-162, doi:10.1007/s12016-007-8039-9 (2008).
106 Worlitzsch, D. *et al.* Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. *J Clin Invest* **109**, 317-325, doi:10.1172/JCI13870 (2002).
107 Harris, W. T. *et al.* Plasma TGF-beta(1) in pediatric cystic fibrosis: potential biomarker of lung disease and response to therapy. *Pediatr Pulmonol* **46**, 688-695, doi:10.1002/ppul.21430 (2011).
108 Wellenstein, M. D. & de Visser, K. E. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. *Immunity* **48**, 399-416, doi:10.1016/j.immuni.2018.03.004 (2018).
109 Dai, Y. *et al.* Copy number gain of ZEB1 mediates a double-negative feedback loop with miR-33a-5p that regulates EMT and bone metastasis of prostate cancer dependent on TGF-beta signaling. *Theranostics* **9**, 6063-6079, doi:10.7150/thno.36735 (2019).
110 Berenguer, J. & Celia-Terrassa, T. Cell memory of epithelial-mesenchymal plasticity in cancer. *Curr Opin Cell Biol* **69**, 103-110, doi:10.1016/j.ceb.2021.01.001 (2021).
111 Ewing, J. A. VII. On the production of transient electric currents in iron and steel conductors by twisting them when magnetised or by magnetising them when twisted. *Proceedings of the Royal Society of London* **33**, 21-23 (1882).
112 Ferrell, J. E., Jr. Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. *Curr Opin Cell Biol* **14**, 140-148, doi:10.1016/s0955-0674(02)00314-9 (2002).
113 Gregory, P. A. *et al.* An autocrine TGF-beta/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial-mesenchymal transition. *Mol Biol Cell* **22**, 1686-1698, doi:10.1091/mbc.E11-02-0103 (2011).
114 Jain, P. *et al.* Epigenetic memory acquired during long-term EMT induction governs the recovery to the epithelial state. *J R Soc Interface* **20**, 20220627, doi:10.1098/rsif.2022.0627 (2023).
115 Stylianou, N. *et al.* A molecular portrait of epithelial-mesenchymal plasticity in prostate cancer associated with clinical outcome. *Oncogene* **38**, 913-934, doi:10.1038/s41388-018-0488-5 (2019).
116 Celia-Terrassa, T. *et al.* Hysteresis control of epithelial-mesenchymal transition dynamics conveys a distinct program with enhanced metastatic ability. *Nat Commun* **9**, 5005, doi:10.1038/s41467-018-07538-7 (2018).
117 Schmidt, J. M. *et al.* Stem-cell-like properties and epithelial plasticity arise as stable traits after transient Twist1 activation. *Cell Rep* **10**, 131-139, doi:10.1016/j.celrep.2014.12.032 (2015).
118 Sarrio, D., Franklin, C. K., Mackay, A., Reis-Filho, J. S. & Isacke, C. M. Epithelial and mesenchymal subpopulations within normal basal breast cell lines exhibit distinct stem cell/progenitor properties. *Stem Cells* **30**, 292-303, doi:10.1002/stem.791 (2012).
119 Harner-Foreman, N. *et al.* A novel spontaneous model of epithelial-mesenchymal transition (EMT) using a primary prostate cancer derived cell line demonstrating distinct stem-like characteristics. *Sci Rep* **7**, 40633, doi:10.1038/srep40633 (2017).
120 Ruscetti, M. *et al.* HDAC inhibition impedes epithelial-mesenchymal plasticity and suppresses metastatic, castration-resistant prostate cancer. *Oncogene* **35**, 3781-3795, doi:10.1038/onc.2015.444 (2016).

121 Mathis, R. A., Sokol, E. S. & Gupta, P. B. Cancer cells exhibit clonal diversity in phenotypic plasticity. *Open Biol* **7**, doi:10.1098/rsob.160283 (2017).

122 Gupta, P. B. *et al.* Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* **146**, 633-644, doi:10.1016/j.cell.2011.07.026 (2011).

123 Bhatia, S. *et al.* Interrogation of Phenotypic Plasticity between Epithelial and Mesenchymal States in Breast Cancer. *J Clin Med* **8**, doi:10.3390/jcm8060893 (2019).

124 Mani, S. A. *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* **133**, 704-715, doi:10.1016/j.cell.2008.03.027 (2008).

125 Neelakantan, D. *et al.* EMT cells increase breast cancer metastasis via paracrine GLI activation in neighbouring tumour cells. *Nat Commun* **8**, 15773, doi:10.1038/ncomms15773 (2017).

126 Lan, L. *et al.* GREM1 is required to maintain cellular heterogeneity in pancreatic cancer. *Nature*, doi:10.1038/s41586-022-04888-7 (2022).

127 Boareto, M. *et al.* Notch-Jagged signalling can give rise to clusters of cells exhibiting a hybrid epithelial/mesenchymal phenotype. *J R Soc Interface* **13**, doi:10.1098/rsif.2015.1106 (2016).

128 Jain, P., Bhatia, S., Thompson, E. W. & Jolly, M. K. Population Dynamics of Epithelial-Mesenchymal Heterogeneity in Cancer Cells. *Biomolecules* **12**, doi:10.3390/biom12030348 (2022).

129 Tripathi, S., Chakraborty, P., Levine, H. & Jolly, M. K. A mechanism for epithelial-mesenchymal heterogeneity in a population of cancer cells. *PLoS Comput Biol* **16**, e1007619, doi:10.1371/journal.pcbi.1007619 (2020).

130 Elowitz, M. B., Levine, A. J., Siggia, E. D. & Swain, P. S. Stochastic gene expression in a single cell. *Science* **297**, 1183-1186, doi:10.1126/science.1070919 (2002).

131 McAdams, H. H. & Arkin, A. Stochastic mechanisms in gene expression. *Proc Natl Acad Sci U S A* **94**, 814-819, doi:10.1073/pnas.94.3.814 (1997).

132 Wellens, T., Shatokhin, V. & Buchleitner, A. Stochastic resonance. *Reports on progress in physics* **67**, 45 (2003).

133 Losick, R. & Desplan, C. Stochasticity and cell fate. *Science* **320**, 65-68, doi:10.1126/science.1147888 (2008).

134 Jia, W. *et al.* Epigenetic feedback and stochastic partitioning during cell division can drive resistance to EMT. *Oncotarget* **11**, 2611-2624, doi:10.18632/oncotarget.27651 (2020).

135 Desai, R. V. *et al.* A DNA repair pathway can regulate transcriptional noise to promote cell fate transitions. *Science* **373**, doi:10.1126/science.abc6506 (2021).

136 Wang, Q., Holmes, W. R., Sosnik, J., Schilling, T. & Nie, Q. Cell Sorting and Noise-Induced Cell Plasticity Coordinate to Sharpen Boundaries between Gene Expression Domains. *PLoS Comput Biol* **13**, e1005307, doi:10.1371/journal.pcbi.1005307 (2017).

137 Matak, A. *et al.* Stochastic phenotype switching leads to intratumor heterogeneity in human liver cancer. *Hepatology* **68**, 933-948, doi:10.1002/hep.29679 (2018).

138 Sosnik, J. *et al.* Noise modulation in retinoic acid signaling sharpens segmental boundaries of gene expression in the embryonic zebrafish hindbrain. *Elife* **5**, e14034, doi:10.7554/eLife.14034 (2016).

139 Zhang, L. *et al.* Noise drives sharpening of gene expression boundaries in the zebrafish hindbrain. *Mol Syst Biol* **8**, 613, doi:10.1038/msb.2012.45 (2012).

140 Chang, H. H., Hemberg, M., Barahona, M., Ingber, D. E. & Huang, S. Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. *Nature* **453**, 544-547, doi:10.1038/nature06965 (2008).

141 Eldar, A. & Elowitz, M. B. Functional roles for noise in genetic circuits. *Nature* **467**, 167-173, doi:10.1038/nature09326 (2010).

142 Pujadas, E. & Feinberg, A. P. Regulated noise in the epigenetic landscape of development and disease. *Cell* **148**, 1123-1131, doi:10.1016/j.cell.2012.02.045 (2012).

143 Ozbudak, E. M., Thattai, M., Kurtser, I., Grossman, A. D. & van Oudenaarden, A. Regulation of noise in the expression of a single gene. *Nat Genet* **31**, 69-73, doi:10.1038/ng869 (2002).
144 Battich, N., Stoeger, T. & Pelkmans, L. Control of Transcript Variability in Single Mammalian Cells. *Cell* **163**, 1596-1610, doi:10.1016/j.cell.2015.11.018 (2015).
145 Arias, A. M. & Hayward, P. Filtering transcriptional noise during development: concepts and mechanisms. *Nat Rev Genet* **7**, 34-44, doi:10.1038/nrg1750 (2006).
146 Bahar, R. *et al.* Increased cell-to-cell variation in gene expression in ageing mouse heart. *Nature* **441**, 1011-1014, doi:10.1038/nature04844 (2006).
147 Kussell, E. & Leibler, S. Phenotypic diversity, population growth, and information in fluctuating environments. *Science* **309**, 2075-2078, doi:10.1126/science.1114383 (2005).
148 Acar, M., Mettetal, J. T. & van Oudenaarden, A. Stochastic switching as a survival strategy in fluctuating environments. *Nat Genet* **40**, 471-475, doi:10.1038/ng.110 (2008).
149 Suter, D. M. *et al.* Mammalian genes are transcribed with widely different bursting kinetics. *Science* **332**, 472-474, doi:10.1126/science.1198817 (2011).
150 Urban, E. A. & Johnston, R. J., Jr. Buffering and Amplifying Transcriptional Noise During Cell Fate Specification. *Front Genet* **9**, 591, doi:10.3389/fgene.2018.00591 (2018).
151 Becskei, A. & Serrano, L. Engineering stability in gene networks by autoregulation. *Nature* **405**, 590-593, doi:10.1038/35014651 (2000).
152 Stoeger, T., Battich, N. & Pelkmans, L. Passive Noise Filtering by Cellular Compartmentalization. *Cell* **164**, 1151-1161, doi:10.1016/j.cell.2016.02.005 (2016).
153 Klosin, A. *et al.* Phase separation provides a mechanism to reduce noise in cells. *Science* **367**, 464-468, doi:10.1126/science.aav6691 (2020).
154 Saunders, T. E. *et al.* Noise reduction in the intracellular pom1p gradient by a dynamic clustering mechanism. *Dev Cell* **22**, 558-572, doi:10.1016/j.devcel.2012.01.001 (2012).
155 Brennan, C. M. *et al.* Protein aggregation mediates stoichiometry of protein complexes in aneuploid cells. *Genes Dev* **33**, 1031-1047, doi:10.1101/gad.327494.119 (2019).
156 Kang, E., Seo, J., Yoon, H. & Cho, S. The Post-Translational Regulation of Epithelial-Mesenchymal Transition-Inducing Transcription Factors in Cancer Metastasis. *Int J Mol Sci* **22**, doi:10.3390/ijms22073591 (2021).
157 Liu, Y. *et al.* Competitive endogenous RNA is an intrinsic component of EMT regulatory circuits and modulates EMT. *Nat Commun* **10**, 1637, doi:10.1038/s41467-019-09649-1 (2019).
158 Salmena, L., Poliseno, L., Tay, Y., Kats, L. & Pandolfi, P. P. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146**, 353-358, doi:10.1016/j.cell.2011.07.014 (2011).
159 Cheng, J. T. *et al.* Insights into Biological Role of LncRNAs in Epithelial-Mesenchymal Transition. *Cells* **8**, doi:10.3390/cells8101178 (2019).
160 O'Brien, S. J. *et al.* Long non-coding RNA (lncRNA) and epithelial-mesenchymal transition (EMT) in colorectal cancer: a systematic review. *Cancer Biol Ther* **21**, 769-781, doi:10.1080/15384047.2020.1794239 (2020).
161 Boulay, J. L., Dennefeld, C. & Alberga, A. The Drosophila developmental gene snail encodes a protein with nucleic acid binding fingers. *Nature* **330**, 395-398, doi:10.1038/330395a0 (1987).
162 Thisse, B., Stoetzel, C., Gorostiza-Thisse, C. & Perrin-Schmitt, F. Sequence of the twist gene and nuclear localization of its protein in endomesodermal cells of early Drosophila embryos. *EMBO J* **7**, 2175-2183, doi:10.1002/j.1460-2075.1988.tb03056.x (1988).
163 Nieto, M. A., Bennett, M. F., Sargent, M. G. & Wilkinson, D. G. Cloning and developmental expression of Sna, a murine homologue of the Drosophila snail gene. *Development* **116**, 227-237, doi:10.1242/dev.116.1.227 (1992).
164 Funahashi, J., Sekido, R., Murai, K., Kamachi, Y. & Kondoh, H. Delta-crystallin enhancer binding protein delta EF1 is a zinc finger-homeodomain protein implicated in

postgastrulation embryogenesis. *Development* **119**, 433-446, doi:10.1242/dev.119.2.433 (1993).
165 Nieto, M. A., Sargent, M. G., Wilkinson, D. G. & Cooke, J. Control of cell behavior during vertebrate development by Slug, a zinc finger gene. *Science* **264**, 835-839, doi:10.1126/science.7513443 (1994).
166 Verschueren, K. *et al.* SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5'-CACCT sequences in candidate target genes. *J Biol Chem* **274**, 20489-20498, doi:10.1074/jbc.274.29.20489 (1999).
167 Yang, J. *et al.* Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* **117**, 927-939, doi:10.1016/j.cell.2004.06.006 (2004).
168 Savagner, P., Yamada, K. M. & Thiery, J. P. The zinc-finger protein slug causes desmosome dissociation, an initial and necessary step for growth factor-induced epithelial-mesenchymal transition. *J Cell Biol* **137**, 1403-1419, doi:10.1083/jcb.137.6.1403 (1997).
169 Grooteclaes, M. L. & Frisch, S. M. Evidence for a function of CtBP in epithelial gene regulation and anoikis. *Oncogene* **19**, 3823-3828, doi:10.1038/sj.onc.1203721 (2000).
170 Comijn, J. *et al.* The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol Cell* **7**, 1267-1278, doi:10.1016/s1097-2765(01)00260-x (2001).
171 Cano, A. *et al.* The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* **2**, 76-83, doi:10.1038/35000025 (2000).
172 Batlle, E. *et al.* The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* **2**, 84-89, doi:10.1038/35000034 (2000).
173 Oba, S. *et al.* miR-200b precursor can ameliorate renal tubulointerstitial fibrosis. *PLoS One* **5**, e13614, doi:10.1371/journal.pone.0013614 (2010).
174 Lovisa, S. *et al.* Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. *Nat Med* **21**, 998-1009, doi:10.1038/nm.3902 (2015).
175 Kida, Y., Asahina, K., Teraoka, H., Gitelman, I. & Sato, T. Twist relates to tubular epithelial-mesenchymal transition and interstitial fibrogenesis in the obstructed kidney. *J Histochem Cytochem* **55**, 661-673, doi:10.1369/jhc.6A7157.2007 (2007).
176 Grande, M. T. *et al.* Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. *Nat Med* **21**, 989-997, doi:10.1038/nm.3901 (2015).
177 Hajra, K. M., Chen, D. Y. & Fearon, E. R. The SLUG zinc-finger protein represses E-cadherin in breast cancer. *Cancer Res* **62**, 1613-1618 (2002).
178 Vesuna, F., van Diest, P., Chen, J. H. & Raman, V. Twist is a transcriptional repressor of E-cadherin gene expression in breast cancer. *Biochem Biophys Res Commun* **367**, 235-241, doi:10.1016/j.bbrc.2007.11.151 (2008).
179 Lehmann, W. *et al.* ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types. *Nat Commun* **7**, 10498, doi:10.1038/ncomms10498 (2016).
180 Mani, S. A. *et al.* Mesenchyme Forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers. *Proc Natl Acad Sci U S A* **104**, 10069-10074, doi:10.1073/pnas.0703900104 (2007).
181 Hartwell, K. A. *et al.* The Spemann organizer gene, Goosecoid, promotes tumor metastasis. *Proc Natl Acad Sci U S A* **103**, 18969-18974, doi:10.1073/pnas.0608636103 (2006).
182 Wang, X. *et al.* Kruppel-like factor 8 induces epithelial to mesenchymal transition and epithelial cell invasion. *Cancer Res* **67**, 7184-7193, doi:10.1158/0008-5472.CAN-06-4729 (2007).
183 Takano, S. *et al.* Prrx1 isoform switching regulates pancreatic cancer invasion and metastatic colonization. *Genes Dev* **30**, 233-247, doi:10.1101/gad.263327.115 (2016).

184 Ocana, O. H. *et al.* Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. *Cancer Cell* **22**, 709-724, doi:10.1016/j.ccr.2012.10.012 (2012).
185 Tavares, A. L. P., Brown, J. A., Ulrich, E. C., Dvorak, K. & Runyan, R. B. Runx2-I is an Early Regulator of Epithelial-Mesenchymal Cell Transition in the Chick Embryo. *Dev Dyn* **247**, 542-554, doi:10.1002/dvdy.24539 (2018).
186 McCoy, E. L. *et al.* Six1 expands the mouse mammary epithelial stem/progenitor cell pool and induces mammary tumors that undergo epithelial-mesenchymal transition. *J Clin Invest* **119**, 2663-2677, doi:10.1172/JCI37691 (2009).
187 Perez-Moreno, M. A. *et al.* A new role for E12/E47 in the repression of E-cadherin expression and epithelial-mesenchymal transitions. *J Biol Chem* **276**, 27424-27431, doi:10.1074/jbc.M100827200 (2001).
188 Sobrado, V. R. *et al.* The class I bHLH factors E2-2A and E2-2B regulate EMT. *J Cell Sci* **122**, 1014-1024, doi:10.1242/jcs.028241 (2009).
189 Stemmler, M. P., Eccles, R. L., Brabletz, S. & Brabletz, T. Non-redundant functions of EMT transcription factors. *Nat Cell Biol* **21**, 102-112, doi:10.1038/s41556-018-0196-y (2019).
190 Goossens, S., Vandamme, N., Van Vlierberghe, P. & Berx, G. EMT transcription factors in cancer development re-evaluated: Beyond EMT and MET. *Biochim Biophys Acta Rev Cancer* **1868**, 584-591, doi:10.1016/j.bbcan.2017.06.006 (2017).
191 Nieto, M. A. The snail superfamily of zinc-finger transcription factors. *Nat Rev Mol Cell Biol* **3**, 155-166, doi:10.1038/nrm757 (2002).
192 Skrypek, N., Goossens, S., De Smedt, E., Vandamme, N. & Berx, G. Epithelial-to-Mesenchymal Transition: Epigenetic Reprogramming Driving Cellular Plasticity. *Trends Genet* **33**, 943-959, doi:10.1016/j.tig.2017.08.004 (2017).
193 Gregory, P. A. *et al.* The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* **10**, 593-601, doi:10.1038/ncb1722 (2008).
194 Park, S. M., Gaur, A. B., Lengyel, E. & Peter, M. E. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* **22**, 894-907, doi:10.1101/gad.1640608 (2008).
195 Kim, N. H. *et al.* A p53/miRNA-34 axis regulates Snail1-dependent cancer cell epithelial-mesenchymal transition. *J Cell Biol* **195**, 417-433, doi:10.1083/jcb.201103097 (2011).
196 Siemens, H. *et al.* miR-34 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal transitions. *Cell Cycle* **10**, 4256-4271, doi:10.4161/cc.10.24.18552 (2011).
197 Lu, M., Jolly, M. K., Levine, H., Onuchic, J. N. & Ben-Jacob, E. MicroRNA-based regulation of epithelial-hybrid-mesenchymal fate determination. *Proc Natl Acad Sci U S A* **110**, 18144-18149, doi:10.1073/pnas.1318192110 (2013).
198 Zhang, J. *et al.* TGF-beta-induced epithelial-to-mesenchymal transition proceeds through stepwise activation of multiple feedback loops. *Sci Signal* **7**, ra91, doi:10.1126/scisignal.2005304 (2014).
199 Tian, X. J., Zhang, H. & Xing, J. Coupled reversible and irreversible bistable switches underlying TGFbeta-induced epithelial to mesenchymal transition. *Biophys J* **105**, 1079-1089, doi:10.1016/j.bpj.2013.07.011 (2013).
200 Gantier, M. P. *et al.* Analysis of microRNA turnover in mammalian cells following Dicer1 ablation. *Nucleic Acids Res* **39**, 5692-5703, doi:10.1093/nar/gkr148 (2011).
201 Bosson, A. D., Zamudio, J. R. & Sharp, P. A. Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. *Mol Cell* **56**, 347-359, doi:10.1016/j.molcel.2014.09.018 (2014).
202 Jansson, M. D. & Lund, A. H. MicroRNA and cancer. *Mol Oncol* **6**, 590-610, doi:10.1016/j.molonc.2012.09.006 (2012).

203 Zhang, J. *et al.* Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinformatics* **13**, 17-24, doi:10.1016/j.gpb.2015.02.001 (2015).
204 Dragomir, M., Chen, B. & Calin, G. A. Exosomal lncRNAs as new players in cell-to-cell communication. *Transl Cancer Res* **7**, S243-S252, doi:10.21037/tcr.2017.10.46 (2018).
205 Zhang, X. *et al.* Hypoxic BMSC-derived exosomal miRNAs promote metastasis of lung cancer cells via STAT3-induced EMT. *Mol Cancer* **18**, 40, doi:10.1186/s12943-019-0959-5 (2019).
206 Li, Z. *et al.* Tumor-derived exosomal lnc-Sox2ot promotes EMT and stemness by acting as a ceRNA in pancreatic ductal adenocarcinoma. *Oncogene* **37**, 3822-3838, doi:10.1038/s41388-018-0237-9 (2018).
207 Wang, X. *et al.* Melittin-induced long non-coding RNA NONHSAT105177 inhibits proliferation and migration of pancreatic ductal adenocarcinoma. *Cell Death Dis* **9**, 940, doi:10.1038/s41419-018-0965-3 (2018).
208 Pan, L. *et al.* Exosomes-mediated transfer of long noncoding RNA ZFAS1 promotes gastric cancer progression. *J Cancer Res Clin Oncol* **143**, 991-1004, doi:10.1007/s00432-017-2361-2 (2017).
209 Wu, D. M. *et al.* TGF-beta-mediated exosomal lnc-MMP2-2 regulates migration and invasion of lung cancer cells to the vasculature by promoting MMP2 expression. *Cancer Med* **7**, 5118-5129, doi:10.1002/cam4.1758 (2018).
210 Xue, M. *et al.* Hypoxic exosomes facilitate bladder tumor growth and development through transferring long non-coding RNA-UCA1. *Mol Cancer* **16**, 143, doi:10.1186/s12943-017-0714-8 (2017).
211 Javaid, S. *et al.* Dynamic chromatin modification sustains epithelial-mesenchymal transition following inducible expression of Snail-1. *Cell Rep* **5**, 1679-1689, doi:10.1016/j.celrep.2013.11.034 (2013).
212 Graff, J. R., Gabrielson, E., Fujii, H., Baylin, S. B. & Herman, J. G. Methylation patterns of the E-cadherin 5' CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression. *J Biol Chem* **275**, 2727-2732, doi:10.1074/jbc.275.4.2727 (2000).
213 Cheng, C. W. *et al.* Mechanisms of inactivation of E-cadherin in breast carcinoma: modification of the two-hit hypothesis of tumor suppressor gene. *Oncogene* **20**, 3814-3823, doi:10.1038/sj.onc.1204505 (2001).
214 Lombaerts, M. *et al.* E-cadherin transcriptional downregulation by promoter methylation but not mutation is related to epithelial-to-mesenchymal transition in breast cancer cell lines. *Br J Cancer* **94**, 661-671, doi:10.1038/sj.bjc.6602996 (2006).
215 Chen, Y., Wang, K., Qian, C. N. & Leach, R. DNA methylation is associated with transcription of Snail and Slug genes. *Biochem Biophys Res Commun* **430**, 1083-1090, doi:10.1016/j.bbrc.2012.12.034 (2013).
216 Galvan, J. A. *et al.* TWIST1 and TWIST2 promoter methylation and protein expression in tumor stroma influence the epithelial-mesenchymal transition-like tumor budding phenotype in colorectal cancer. *Oncotarget* **6**, 874-885, doi:10.18632/oncotarget.2716 (2015).
217 Alghamian, Y., Soukkarieh, C., Abbady, A. Q. & Murad, H. Investigation of role of CpG methylation in some epithelial mesenchymal transition gene in a chemoresistant ovarian cancer cell line. *Sci Rep* **12**, 7494, doi:10.1038/s41598-022-11634-6 (2022).
218 Vrba, L. *et al.* Role for DNA methylation in the regulation of miR-200c and miR-141 expression in normal and cancer cells. *PLoS One* **5**, e8697, doi:10.1371/journal.pone.0008697 (2010).
219 Wiklund, E. D. *et al.* Coordinated epigenetic repression of the miR-200 family and miR-205 in invasive bladder cancer. *Int J Cancer* **128**, 1327-1334, doi:10.1002/ijc.25461 (2011).

220 Davalos, V. *et al.* Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis. *Oncogene* **31**, 2062-2074, doi:10.1038/onc.2011.383 (2012).
221 Tellez, C. S. *et al.* EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. *Cancer Res* **71**, 3087-3097, doi:10.1158/0008-5472.CAN-10-3035 (2011).
222 Dang, T. T., Esparza, M. A., Maine, E. A., Westcott, J. M. & Pearson, G. W. DeltaNp63alpha Promotes Breast Cancer Cell Motility through the Selective Activation of Components of the Epithelial-to-Mesenchymal Transition Program. *Cancer Res* **75**, 3925-3935, doi:10.1158/0008-5472.CAN-14-3363 (2015).
223 Abell, A. N. *et al.* MAP3K4/CBP-regulated H2B acetylation controls epithelial-mesenchymal transition in trophoblast stem cells. *Cell Stem Cell* **8**, 525-537, doi:10.1016/j.stem.2011.03.008 (2011).
224 Warzecha, C. C., Sato, T. K., Nabet, B., Hogenesch, J. B. & Carstens, R. P. ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing. *Mol Cell* **33**, 591-601, doi:10.1016/j.molcel.2009.01.025 (2009).
225 Segelle, A. *et al.* Histone marks regulate the epithelial-to-mesenchymal transition via alternative splicing. *Cell Rep* **38**, 110357, doi:10.1016/j.celrep.2022.110357 (2022).
226 Sahu, S. K. *et al.* A complex epigenome-splicing crosstalk governs epithelial-to-mesenchymal transition in metastasis and brain development. *Nat Cell Biol* **24**, 1265-1277, doi:10.1038/s41556-022-00971-3 (2022).
227 Gomes, A. P. *et al.* Dynamic Incorporation of Histone H3 Variants into Chromatin Is Essential for Acquisition of Aggressive Traits and Metastatic Colonization. *Cancer Cell* **36**, 402-417 e413, doi:10.1016/j.ccell.2019.08.006 (2019).
228 Hodge, D. Q., Cui, J., Gamble, M. J. & Guo, W. Histone Variant MacroH2A1 Plays an Isoform-Specific Role in Suppressing Epithelial-Mesenchymal Transition. *Sci Rep* **8**, 841, doi:10.1038/s41598-018-19364-4 (2018).
229 Dumont, N. *et al.* Sustained induction of epithelial to mesenchymal transition activates DNA methylation of genes silenced in basal-like breast cancers. *Proc Natl Acad Sci U S A* **105**, 14867-14872, doi:10.1073/pnas.0807146105 (2008).
230 Galle, E. *et al.* DNA methylation-driven EMT is a common mechanism of resistance to various therapeutic agents in cancer. *Clin Epigenetics* **12**, 27, doi:10.1186/s13148-020-0821-z (2020).
231 Jia, W., Deshmukh, A., Mani, S. A., Jolly, M. K. & Levine, H. A possible role for epigenetic feedback regulation in the dynamics of the epithelial-mesenchymal transition (EMT). *Phys Biol* **16**, 066004, doi:10.1088/1478-3975/ab34df (2019).
232 Yuan, S. *et al.* Global Regulation of the Histone Mark H3K36me2 Underlies Epithelial Plasticity and Metastatic Progression. *Cancer Discov* **10**, 854-871, doi:10.1158/2159-8290.CD-19-1299 (2020).
233 Morin, C., Moyret-Lalle, C., Mertani, H. C., Diaz, J. J. & Marcel, V. Heterogeneity and dynamic of EMT through the plasticity of ribosome and mRNA translation. *Biochim Biophys Acta Rev Cancer* **1877**, 188718, doi:10.1016/j.bbcan.2022.188718 (2022).
234 Willig, T. N. *et al.* Mutations in ribosomal protein S19 gene and Diamond Blackfan anemia: Wide variations in phenotypic expression. *Blood* **94**, 4294-4306 (1999).
235 Boria, I. *et al.* The ribosomal basis of Diamond-Blackfan Anemia: mutation and database update. *Hum Mutat* **31**, 1269-1279, doi:10.1002/humu.21383 (2010).
236 Lipton, J. M. & Ellis, S. R. Diamond-Blackfan anemia: diagnosis, treatment, and molecular pathogenesis. *Hematol Oncol Clin North Am* **23**, 261-282, doi:10.1016/j.hoc.2009.01.004 (2009).
237 Dauwerse, J. G. *et al.* Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome. *Nat Genet* **43**, 20-22, doi:10.1038/ng.724 (2011).

238 Valdez, B. C., Henning, D., So, R. B., Dixon, J. & Dixon, M. J. The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor. *Proc Natl Acad Sci U S A* **101**, 10709-10714, doi:10.1073/pnas.0402492101 (2004).
239 Wielosz, E., Dryglewska, M. & Majdan, M. Clinical consequences of the presence of anti-RNA Pol III antibodies in systemic sclerosis. *Postepy Dermatol Alergol* **37**, 909-914, doi:10.5114/ada.2020.102107 (2020).
240 Ochs, R. L., Lischwe, M. A., Spohn, W. H. & Busch, H. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. *Biol Cell* **54**, 123-133, doi:10.1111/j.1768-322x.1985.tb00387.x (1985).
241 Arnett, F. C. *et al.* Autoantibodies to fibrillarin in systemic sclerosis (scleroderma). An immunogenetic, serologic, and clinical analysis. *Arthritis Rheum* **39**, 1151-1160, doi:10.1002/art.1780390712 (1996).
242 Bassler, J. & Hurt, E. Eukaryotic Ribosome Assembly. *Annu Rev Biochem* **88**, 281-306, doi:10.1146/annurev-biochem-013118-110817 (2019).
243 Benyelles, M. *et al.* NHP2 deficiency impairs rRNA biogenesis and causes pulmonary fibrosis and Hoyeraal-Hreidarsson syndrome. *Hum Mol Genet* **29**, 907-922, doi:10.1093/hmg/ddaa011 (2020).
244 Freed, E. F. & Baserga, S. J. The C-terminus of Utp4, mutated in childhood cirrhosis, is essential for ribosome biogenesis. *Nucleic Acids Res* **38**, 4798-4806, doi:10.1093/nar/gkq185 (2010).
245 Freed, E. F., Prieto, J. L., McCann, K. L., McStay, B. & Baserga, S. J. NOL11, implicated in the pathogenesis of North American Indian childhood cirrhosis, is required for pre-rRNA transcription and processing. *PLoS Genet* **8**, e1002892, doi:10.1371/journal.pgen.1002892 (2012).
246 Thorolfsdottir, R. B. *et al.* Coding variants in RPL3L and MYZAP increase risk of atrial fibrillation. *Commun Biol* **1**, 68, doi:10.1038/s42003-018-0068-9 (2018).
247 Prakash, V. *et al.* Ribosome biogenesis during cell cycle arrest fuels EMT in development and disease. *Nat Commun* **10**, 2110, doi:10.1038/s41467-019-10100-8 (2019).
248 Hou, Y. *et al.* Airway Basal Cells Mediate Hypoxia-Induced EMT by Increasing Ribosome Biogenesis. *Front Pharmacol* **12**, 783946, doi:10.3389/fphar.2021.783946 (2021).
249 Ito, N. *et al.* Ribosome Incorporation into Somatic Cells Promotes Lineage Transdifferentiation towards Multipotency. *Sci Rep* **8**, 1634, doi:10.1038/s41598-018-20057-1 (2018).
250 Kudo, M. *et al.* Ribosome Incorporation Induces EMT-like Phenomenon with Cell Cycle Arrest in Human Breast Cancer Cell. *Cells Tissues Organs* **211**, 212-221, doi:10.1159/000513908 (2022).
251 Mili, S., Moissoglu, K. & Macara, I. G. Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions. *Nature* **453**, 115-119, doi:10.1038/nature06888 (2008).
252 Mardakheh, F. K. *et al.* Global Analysis of mRNA, Translation, and Protein Localization: Local Translation Is a Key Regulator of Cell Protrusions. *Dev Cell* **35**, 344-357, doi:10.1016/j.devcel.2015.10.005 (2015).
253 Wang, X. *et al.* Methyltransferase like 13 mediates the translation of Snail in head and neck squamous cell carcinoma. *Int J Oral Sci* **13**, 26, doi:10.1038/s41368-021-00130-8 (2021).
254 Dermit, M. *et al.* Subcellular mRNA Localization Regulates Ribosome Biogenesis in Migrating Cells. *Dev Cell* **55**, 298-313 e210, doi:10.1016/j.devcel.2020.10.006 (2020).
255 Lin, X. *et al.* RNA m(6)A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. *Nat Commun* **10**, 2065, doi:10.1038/s41467-019-09865-9 (2019).
256 Chaudhury, A. *et al.* CELF1 is a central node in post-transcriptional regulatory programmes underlying EMT. *Nat Commun* **7**, 13362, doi:10.1038/ncomms13362 (2016).

257 Evdokimova, V. *et al.* Translational activation of snail1 and other developmentally regulated transcription factors by YB-1 promotes an epithelial-mesenchymal transition. *Cancer Cell* **15**, 402-415, doi:10.1016/j.ccr.2009.03.017 (2009).
258 Gulhati, P. *et al.* mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Res* **71**, 3246-3256, doi:10.1158/0008-5472.CAN-10-4058 (2011).
259 Cai, W., Ye, Q. & She, Q. B. Loss of 4E-BP1 function induces EMT and promotes cancer cell migration and invasion via cap-dependent translational activation of snail. *Oncotarget* **5**, 6015-6027, doi:10.18632/oncotarget.2109 (2014).
260 Azmi, A. S. *et al.* Targeting the Nuclear Export Protein XPO1/CRM1 Reverses Epithelial to Mesenchymal Transition. *Sci Rep* **5**, 16077, doi:10.1038/srep16077 (2015).
261 Kang, H., Kim, H., Lee, S., Youn, H. & Youn, B. Role of Metabolic Reprogramming in Epithelial(-)Mesenchymal Transition (EMT). *Int J Mol Sci* **20**, doi:10.3390/ijms20082042 (2019).
262 Georgakopoulos-Soares, I., Chartoumpekis, D. V., Kyriazopoulou, V. & Zaravinos, A. EMT Factors and Metabolic Pathways in Cancer. *Front Oncol* **10**, 499, doi:10.3389/fonc.2020.00499 (2020).
263 Sciacovelli, M. & Frezza, C. Metabolic reprogramming and epithelial-to-mesenchymal transition in cancer. *FEBS J* **284**, 3132-3144, doi:10.1111/febs.14090 (2017).
264 Hua, W., Ten Dijke, P., Kostidis, S., Giera, M. & Hornsveld, M. TGFbeta-induced metabolic reprogramming during epithelial-to-mesenchymal transition in cancer. *Cell Mol Life Sci* **77**, 2103-2123, doi:10.1007/s00018-019-03398-6 (2020).
265 Cha, Y. H., Yook, J. I., Kim, H. S. & Kim, N. H. Catabolic metabolism during cancer EMT. *Arch Pharm Res* **38**, 313-320, doi:10.1007/s12272-015-0567-x (2015).
266 Swinnen, J. V., Brusselmans, K. & Verhoeven, G. Increased lipogenesis in cancer cells: new players, novel targets. *Curr Opin Clin Nutr Metab Care* **9**, 358-365, doi:10.1097/01.mco.0000232894.28674.30 (2006).
267 Kuhajda, F. P. Fatty acid synthase and cancer: new application of an old pathway. *Cancer Res* **66**, 5977-5980, doi:10.1158/0008-5472.CAN-05-4673 (2006).
268 Zhu, Y. *et al.* Posttranslational control of lipogenesis in the tumor microenvironment. *J Hematol Oncol* **15**, 120, doi:10.1186/s13045-022-01340-1 (2022).
269 Singh, R., Yadav, V., Kumar, S. & Saini, N. MicroRNA-195 inhibits proliferation, invasion and metastasis in breast cancer cells by targeting FASN, HMGCR, ACACA and CYP27B1. *Sci Rep* **5**, 17454, doi:10.1038/srep17454 (2015).
270 Gong, J. *et al.* Inhibition of FASN suppresses migration, invasion and growth in hepatoma carcinoma cells by deregulating the HIF-1alpha/IGFBP1 pathway. *Int J Oncol* **50**, 883-892, doi:10.3892/ijo.2017.3867 (2017).
271 Jiang, L. *et al.* Up-regulated FASN expression promotes transcoelomic metastasis of ovarian cancer cell through epithelial-mesenchymal transition. *Int J Mol Sci* **15**, 11539-11554, doi:10.3390/ijms150711539 (2014).
272 Sun, L. *et al.* Small interfering RNA-mediated knockdown of fatty acid synthase attenuates the proliferation and metastasis of human gastric cancer cells via the mTOR/Gli1 signaling pathway. *Oncol Lett* **16**, 594-602, doi:10.3892/ol.2018.8648 (2018).
273 Yang, L. *et al.* A FASN-TGF-beta1-FASN regulatory loop contributes to high EMT/metastatic potential of cisplatin-resistant non-small cell lung cancer. *Oncotarget* **7**, 55543-55554, doi:10.18632/oncotarget.10837 (2016).
274 Gonzalez-Guerrico, A. M. *et al.* Suppression of endogenous lipogenesis induces reversion of the malignant phenotype and normalized differentiation in breast cancer. *Oncotarget* **7**, 71151-71168, doi:10.18632/oncotarget.9463 (2016).
275 Chen, Y. *et al.* ZEB1 Regulates Multiple Oncogenic Components Involved in Uveal Melanoma Progression. *Sci Rep* **7**, 45, doi:10.1038/s41598-017-00079-x (2017).

276 Hung, C. M. *et al.* Osthole suppresses hepatocyte growth factor (HGF)-induced epithelial-mesenchymal transition via repression of the c-Met/Akt/mTOR pathway in human breast cancer cells. *J Agric Food Chem* **59**, 9683-9690, doi:10.1021/jf2021489 (2011).
277 Angelucci, C. *et al.* Epithelial-stromal interactions in human breast cancer: effects on adhesion, plasma membrane fluidity and migration speed and directness. *PLoS One* **7**, e50804, doi:10.1371/journal.pone.0050804 (2012).
278 Edmond, V. *et al.* Downregulation of ceramide synthase-6 during epithelial-to-mesenchymal transition reduces plasma membrane fluidity and cancer cell motility. *Oncogene* **34**, 996-1005, doi:10.1038/onc.2014.55 (2015).
279 Taraboletti, G. *et al.* Membrane fluidity affects tumor-cell motility, invasion and lung-colonizing potential. *Int J Cancer* **44**, 707-713, doi:10.1002/ijc.2910440426 (1989).
280 Zhao, W. *et al.* Candidate Antimetastasis Drugs Suppress the Metastatic Capacity of Breast Cancer Cells by Reducing Membrane Fluidity. *Cancer Res* **76**, 2037-2049, doi:10.1158/0008-5472.CAN-15-1970 (2016).
281 Wang, Y. *et al.* NNMT contributes to high metastasis of triple negative breast cancer by enhancing PP2A/MEK/ERK/c-Jun/ABCA1 pathway mediated membrane fluidity. *Cancer Lett* **547**, 215884, doi:10.1016/j.canlet.2022.215884 (2022).
282 Eiriksson, F. F. *et al.* Altered plasmalogen content and fatty acid saturation following epithelial to mesenchymal transition in breast epithelial cell lines. *Int J Biochem Cell Biol* **103**, 99-104, doi:10.1016/j.biocel.2018.08.003 (2018).
283 Yu, H., Duan, P., Zhu, H. & Rao, D. miR-613 inhibits bladder cancer proliferation and migration through targeting SphK1. *Am J Transl Res* **9**, 1213-1221 (2017).
284 Fan, Z., Jiang, H., Wang, Z. & Qu, J. Atorvastatin partially inhibits the epithelial-mesenchymal transition in A549 cells induced by TGF-beta1 by attenuating the upregulation of SphK1. *Oncol Rep* **36**, 1016-1022, doi:10.3892/or.2016.4897 (2016).
285 Long, J., Xie, Y., Yin, J., Lu, W. & Fang, S. SphK1 promotes tumor cell migration and invasion in colorectal cancer. *Tumour Biol* **37**, 6831-6836, doi:10.1007/s13277-015-4542-4 (2016).
286 Liu, H., Ma, Y., He, H. W., Zhao, W. L. & Shao, R. G. SPHK1 (sphingosine kinase 1) induces epithelial-mesenchymal transition by promoting the autophagy-linked lysosomal degradation of CDH1/E-cadherin in hepatoma cells. *Autophagy* **13**, 900-913, doi:10.1080/15548627.2017.1291479 (2017).
287 Xu, C. Y. *et al.* SphK1 modulates cell migration and EMT-related marker expression by regulating the expression of p-FAK in colorectal cancer cells. *Int J Mol Med* **39**, 1277-1284, doi:10.3892/ijmm.2017.2921 (2017).
288 Meshcheryakova, A. *et al.* Exploring the role of sphingolipid machinery during the epithelial to mesenchymal transition program using an integrative approach. *Oncotarget* **7**, 22295-22323, doi:10.18632/oncotarget.7947 (2016).
289 Zeng, Y. *et al.* Sphingosine-1-phosphate induced epithelial-mesenchymal transition of hepatocellular carcinoma via an MMP-7/ syndecan-1/TGF-beta autocrine loop. *Oncotarget* **7**, 63324-63337, doi:10.18632/oncotarget.11450 (2016).
290 Ishizawa, S. *et al.* Sphingosine-1-phosphate induces differentiation of cultured renal tubular epithelial cells under Rho kinase activation via the S1P2 receptor. *Clin Exp Nephrol* **18**, 844-852, doi:10.1007/s10157-014-0933-x (2014).
291 Zeng, Y. E., Yao, X. H., Yan, Z. P., Liu, J. X. & Liu, X. H. Potential signaling pathway involved in sphingosine-1-phosphate-induced epithelial-mesenchymal transition in cancer. *Oncol Lett* **12**, 379-382, doi:10.3892/ol.2016.4661 (2016).
292 Fajardo, V. A., McMeekin, L. & LeBlanc, P. J. Influence of phospholipid species on membrane fluidity: a meta-analysis for a novel phospholipid fluidity index. *J Membr Biol* **244**, 97-103, doi:10.1007/s00232-011-9401-7 (2011).

293 Ruiz, M. *et al.* Sphingosine 1-phosphate mediates adiponectin receptor signaling essential for lipid homeostasis and embryogenesis. *Nat Commun* **13**, 7162, doi:10.1038/s41467-022-34931-0 (2022).
294 Lu, P., White-Gilbertson, S., Nganga, R., Kester, M. & Voelkel-Johnson, C. Expression of the SNAI2 transcriptional repressor is regulated by C(16)-ceramide. *Cancer Biol Ther* **20**, 922-930, doi:10.1080/15384047.2019.1579962 (2019).
295 Prijic, S. & Chang, J. T. ABCA1 Expression Is Upregulated in an EMT in Breast Cancer Cell Lines via MYC-Mediated De-Repression of Its Proximal Ebox Element. *Biomedicines* **10**, doi:10.3390/biomedicines10030581 (2022).
296 Alonso, A. & Goni, F. M. The Physical Properties of Ceramides in Membranes. *Annu Rev Biophys* **47**, 633-654, doi:10.1146/annurev-biophys-070317-033309 (2018).
297 van Blitterswijk, W. J., van der Meer, B. W. & Hilkmann, H. Quantitative contributions of cholesterol and the individual classes of phospholipids and their degree of fatty acyl (un)saturation to membrane fluidity measured by fluorescence polarization. *Biochemistry* **26**, 1746-1756, doi:10.1021/bi00380a038 (1987).
298 Dalmau, N., Jaumot, J., Tauler, R. & Bedia, C. Epithelial-to-mesenchymal transition involves triacylglycerol accumulation in DU145 prostate cancer cells. *Mol Biosyst* **11**, 3397-3406, doi:10.1039/c5mb00413f (2015).
299 Lin, S. J., Yang, D. R., Li, G. & Chang, C. TR4 Nuclear Receptor Different Roles in Prostate Cancer Progression. *Front Endocrinol (Lausanne)* **6**, 78, doi:10.3389/fendo.2015.00078 (2015).
300 Xu, X. *et al.* VSP-17 suppresses the migration and invasion of triple-negative breast cancer cells through inhibition of the EMT process via the PPARgamma/AMPK signaling pathway. *Oncol Rep* **45**, 975-986, doi:10.3892/or.2020.7916 (2021).
301 Reka, A. K. *et al.* Peroxisome proliferator-activated receptor-gamma activation inhibits tumor metastasis by antagonizing Smad3-mediated epithelial-mesenchymal transition. *Mol Cancer Ther* **9**, 3221-3232, doi:10.1158/1535-7163.MCT-10-0570 (2010).
302 Liu, J. *et al.* Bergenin inhibits bladder cancer progression via activating the PPARgamma/PTEN/Akt signal pathway. *Drug Dev Res* **82**, 278-286, doi:10.1002/ddr.21751 (2021).
303 Di Gregorio, J. *et al.* Role of glycogen synthase kinase-3beta and PPAR-gamma on epithelial-to-mesenchymal transition in DSS-induced colorectal fibrosis. *PLoS One* **12**, e0171093, doi:10.1371/journal.pone.0171093 (2017).
304 Wu, C. T., Wang, C. C., Huang, L. C., Liu, S. H. & Chiang, C. K. Plasticizer Di-(2-Ethylhexyl)Phthalate Induces Epithelial-to-Mesenchymal Transition and Renal Fibrosis In Vitro and In Vivo. *Toxicol Sci* **164**, 363-374, doi:10.1093/toxsci/kfy094 (2018).
305 Wang, Q. *et al.* Integrin beta4 in EMT: an implication of renal diseases. *Int J Clin Exp Med* **8**, 6967-6976 (2015).
306 Bai, X., Hou, X., Tian, J., Geng, J. & Li, X. CDK5 promotes renal tubulointerstitial fibrosis in diabetic nephropathy via ERK1/2/PPARgamma pathway. *Oncotarget* **7**, 36510-36528, doi:10.18632/oncotarget.9058 (2016).
307 Li, R. *et al.* Curcumin inhibits transforming growth factor-beta1-induced EMT via PPARgamma pathway, not Smad pathway in renal tubular epithelial cells. *PLoS One* **8**, e58848, doi:10.1371/journal.pone.0058848 (2013).
308 Bocca, C. *et al.* Expression of Cox-2 in human breast cancer cells as a critical determinant of epithelial-to-mesenchymal transition and invasiveness. *Expert Opin Ther Targets* **18**, 121-135, doi:10.1517/14728222.2014.860447 (2014).
309 Li, Z. L. *et al.* COX-2 promotes metastasis in nasopharyngeal carcinoma by mediating interactions between cancer cells and myeloid-derived suppressor cells. *Oncoimmunology* **4**, e1044712, doi:10.1080/2162402X.2015.1044712 (2015).

310 Che, D. *et al.* Macrophages induce EMT to promote invasion of lung cancer cells through the IL-6-mediated COX-2/PGE(2)/beta-catenin signalling pathway. *Mol Immunol* **90**, 197-210, doi:10.1016/j.molimm.2017.06.018 (2017).
311 Qin, G. *et al.* Palbociclib inhibits epithelial-mesenchymal transition and metastasis in breast cancer via c-Jun/COX-2 signaling pathway. *Oncotarget* **6**, 41794-41808, doi:10.18632/oncotarget.5993 (2015).
312 Wang, Y. P., Wang, Q. Y., Li, C. H. & Li, X. W. COX-2 inhibition by celecoxib in epithelial ovarian cancer attenuates E-cadherin suppression through reduced Snail nuclear translocation. *Chem Biol Interact* **292**, 24-29, doi:10.1016/j.cbi.2018.06.020 (2018).
313 Xu, Z. *et al.* Lipoxin A4 interferes with embryo implantation via suppression of epithelial-mesenchymal transition. *Am J Reprod Immunol* **81**, e13107, doi:10.1111/aji.13107 (2019).
314 Wu, R. F. *et al.* Lipoxin A(4) Suppresses Estrogen-Induced Epithelial-Mesenchymal Transition via ALXR-Dependent Manner in Endometriosis. *Reprod Sci* **25**, 566-578, doi:10.1177/1933719117718271 (2018).
315 Yang, J. X. *et al.* Lipoxin A(4) ameliorates lipopolysaccharide-induced lung injury through stimulating epithelial proliferation, reducing epithelial cell apoptosis and inhibits epithelial-mesenchymal transition. *Respir Res* **20**, 192, doi:10.1186/s12931-019-1158-z (2019).
316 Rodgers, K., McMahon, B., Mitchell, D., Sadlier, D. & Godson, C. Lipoxin A4 modifies platelet-derived growth factor-induced pro-fibrotic gene expression in human renal mesangial cells. *Am J Pathol* **167**, 683-694, doi:10.1016/S0002-9440(10)62043-3 (2005).
317 Xu, F. *et al.* Lipoxin A(4) and its analog suppress hepatocarcinoma cell epithelial-mesenchymal transition, migration and metastasis via regulating integrin-linked kinase axis. *Prostaglandins Other Lipid Mediat* **137**, 9-19, doi:10.1016/j.prostaglandins.2018.05.007 (2018).
318 Zong, L. *et al.* Lipoxin A4 reverses mesenchymal phenotypes to attenuate invasion and metastasis via the inhibition of autocrine TGF-beta1 signaling in pancreatic cancer. *J Exp Clin Cancer Res* **36**, 181, doi:10.1186/s13046-017-0655-5 (2017).
319 Warburg, O., Wind, F. & Negelein, E. The Metabolism of Tumors in the Body. *J Gen Physiol* **8**, 519-530, doi:10.1085/jgp.8.6.519 (1927).
320 Hamabe, A. *et al.* Role of pyruvate kinase M2 in transcriptional regulation leading to epithelial-mesenchymal transition. *Proc Natl Acad Sci U S A* **111**, 15526-15531, doi:10.1073/pnas.1407717111 (2014).
321 Fan, F. T. *et al.* PKM2 regulates hepatocellular carcinoma cell epithelial-mesenchymal transition and migration upon EGFR activation. *Asian Pac J Cancer Prev* **15**, 1961-1970, doi:10.7314/apjcp.2014.15.5.1961 (2014).
322 Chen, G. *et al.* Deregulation of Hexokinase II Is Associated with Glycolysis, Autophagy, and the Epithelial-Mesenchymal Transition in Tongue Squamous Cell Carcinoma under Hypoxia. *Biomed Res Int* **2018**, 8480762, doi:10.1155/2018/8480762 (2018).
323 Wang, Y. *et al.* LncRNA-p23154 promotes the invasion-metastasis potential of oral squamous cell carcinoma by regulating Glut1-mediated glycolysis. *Cancer Lett* **434**, 172-183, doi:10.1016/j.canlet.2018.07.016 (2018).
324 Yalcin, A. *et al.* 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase-3 is required for transforming growth factor beta1-enhanced invasion of Panc1 cells in vitro. *Biochem Biophys Res Commun* **484**, 687-693, doi:10.1016/j.bbrc.2017.01.178 (2017).
325 Lunetti, P. *et al.* Metabolic reprogramming in breast cancer results in distinct mitochondrial bioenergetics between luminal and basal subtypes. *FEBS J* **286**, 688-709, doi:10.1111/febs.14756 (2019).
326 Zhang, J. *et al.* TGF-beta1 induces epithelial-to-mesenchymal transition via inhibiting mitochondrial functions in A549 cells. *Free Radic Res* **52**, 1432-1444, doi:10.1080/10715762.2018.1500020 (2018).

327 Yi, E. Y., Park, S. Y., Jung, S. Y., Jang, W. J. & Kim, Y. J. Mitochondrial dysfunction induces EMT through the TGF-beta/Smad/Snail signaling pathway in Hep3B hepatocellular carcinoma cells. *Int J Oncol* **47**, 1845-1853, doi:10.3892/ijo.2015.3154 (2015).

328 Guha, M. *et al.* Mitochondrial retrograde signaling induces epithelial-mesenchymal transition and generates breast cancer stem cells. *Oncogene* **33**, 5238-5250, doi:10.1038/onc.2013.467 (2014).

329 Li, X. *et al.* Upregulation of lactate-inducible snail protein suppresses oncogene-mediated senescence through p16(INK4a) inactivation. *J Exp Clin Cancer Res* **37**, 39, doi:10.1186/s13046-018-0701-y (2018).

330 Fiaschi, T. *et al.* Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. *Cancer Res* **72**, 5130-5140, doi:10.1158/0008-5472.CAN-12-1949 (2012).

331 Wang, H. *et al.* TOP1MT deficiency promotes GC invasion and migration via the enhancements of LDHA expression and aerobic glycolysis. *Endocr Relat Cancer* **24**, 565-578, doi:10.1530/ERC-17-0058 (2017).

332 Park, G. B. & Kim, D. TLR4-mediated galectin-1 production triggers epithelial-mesenchymal transition in colon cancer cells through ADAM10- and ADAM17-associated lactate production. *Mol Cell Biochem* **425**, 191-202, doi:10.1007/s11010-016-2873-0 (2017).

333 Corbet, C. *et al.* TGFbeta2-induced formation of lipid droplets supports acidosis-driven EMT and the metastatic spreading of cancer cells. *Nat Commun* **11**, 454, doi:10.1038/s41467-019-14262-3 (2020).

334 Bhattacharya, D., Azambuja, A. P. & Simoes-Costa, M. Metabolic Reprogramming Promotes Neural Crest Migration via Yap/Tead Signaling. *Dev Cell* **53**, 199-211 e196, doi:10.1016/j.devcel.2020.03.005 (2020).

335 Bulusu, V. *et al.* Spatiotemporal Analysis of a Glycolytic Activity Gradient Linked to Mouse Embryo Mesoderm Development. *Dev Cell* **40**, 331-341 e334, doi:10.1016/j.devcel.2017.01.015 (2017).

336 Oginuma, M. *et al.* A Gradient of Glycolytic Activity Coordinates FGF and Wnt Signaling during Elongation of the Body Axis in Amniote Embryos. *Dev Cell* **40**, 342-353 e310, doi:10.1016/j.devcel.2017.02.001 (2017).

337 Youssef, K. K. & Nieto, M. A. Glucose Metabolism Takes Center Stage in Epithelial-Mesenchymal Plasticity. *Dev Cell* **53**, 133-135, doi:10.1016/j.devcel.2020.03.021 (2020).

338 Rossi, M. *et al.* PHGDH heterogeneity potentiates cancer cell dissemination and metastasis. *Nature* **605**, 747-753, doi:10.1038/s41586-022-04758-2 (2022).

339 Schwager, S. C. *et al.* Link between glucose metabolism and epithelial-to-mesenchymal transition drives triple-negative breast cancer migratory heterogeneity. *iScience* **25**, 105190, doi:10.1016/j.isci.2022.105190 (2022).

340 Siddiqui, A. *et al.* Thymidylate synthase maintains the de-differentiated state of triple negative breast cancers. *Cell Death Differ* **26**, 2223-2236, doi:10.1038/s41418-019-0289-6 (2019).

341 Ramesh, V., Brabletz, T. & Ceppi, P. Targeting EMT in Cancer with Repurposed Metabolic Inhibitors. *Trends Cancer* **6**, 942-950, doi:10.1016/j.trecan.2020.06.005 (2020).

342 Luond, F. *et al.* Distinct contributions of partial and full EMT to breast cancer malignancy. *Dev Cell* **56**, 3203-3221 e3211, doi:10.1016/j.devcel.2021.11.006 (2021).

343 Verstappe, J. & Berx, G. A role for partial epithelial-to-mesenchymal transition in enabling stemness in homeostasis and cancer. *Semin Cancer Biol* **90**, 15-28, doi:10.1016/j.semcancer.2023.02.001 (2023).

344 Lambert, A. W. & Weinberg, R. A. Linking EMT programmes to normal and neoplastic epithelial stem cells. *Nat Rev Cancer* **21**, 325-338, doi:10.1038/s41568-021-00332-6 (2021).

345 Yu, M. *et al.* Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* **339**, 580-584, doi:10.1126/science.1228522 (2013).

346 Schliekelman, M. J. *et al.* Molecular portraits of epithelial, mesenchymal, and hybrid States in lung adenocarcinoma and their relevance to survival. *Cancer Res* **75**, 1789-1800, doi:10.1158/0008-5472.CAN-14-2535 (2015).
347 Ruscetti, M., Quach, B., Dadashian, E. L., Mulholland, D. J. & Wu, H. Tracking and Functional Characterization of Epithelial-Mesenchymal Transition and Mesenchymal Tumor Cells during Prostate Cancer Metastasis. *Cancer Res* **75**, 2749-2759, doi:10.1158/0008-5472.CAN-14-3476 (2015).
348 Cook, D. P. & Wrana, J. L. A specialist-generalist framework for epithelial-mesenchymal plasticity in cancer. *Trends Cancer* **8**, 358-368, doi:10.1016/j.trecan.2022.01.014 (2022).
349 Saxena, K., Jolly, M. K. & Balamurugan, K. Hypoxia, partial EMT and collective migration: Emerging culprits in metastasis. *Transl Oncol* **13**, 100845, doi:10.1016/j.tranon.2020.100845 (2020).
350 Quan, Q. *et al.* Cancer stem-like cells with hybrid epithelial/mesenchymal phenotype leading the collective invasion. *Cancer Sci* **111**, 467-476, doi:10.1111/cas.14285 (2020).
351 Biswas, K., Jolly, M. K. & Ghosh, A. Stability and mean residence times for hybrid epithelial/mesenchymal phenotype. *Phys Biol* **16**, 025003, doi:10.1088/1478-3975/aaf7b7 (2019).
352 Jolly, M. K. *et al.* Stability of the hybrid epithelial/mesenchymal phenotype. *Oncotarget* **7**, 27067-27084, doi:10.18632/oncotarget.8166 (2016).
353 Jia, D. *et al.* OVOL guides the epithelial-hybrid-mesenchymal transition. *Oncotarget* **6**, 15436-15448, doi:10.18632/oncotarget.3623 (2015).
354 Bocci, F. *et al.* Numb prevents a complete epithelial-mesenchymal transition by modulating Notch signalling. *J R Soc Interface* **14**, doi:10.1098/rsif.2017.0512 (2017).
355 Subbalakshmi, A. R. *et al.* NFATc Acts as a Non-Canonical Phenotypic Stability Factor for a Hybrid Epithelial/Mesenchymal Phenotype. *Front Oncol* **10**, 553342, doi:10.3389/fonc.2020.553342 (2020).
356 Vilchez Mercedes, S. A. *et al.* Nrf2 Modulates the Hybrid Epithelial/Mesenchymal Phenotype and Notch Signaling During Collective Cancer Migration. *Front Mol Biosci* **9**, 807324, doi:10.3389/fmolb.2022.807324 (2022).
357 Bocci, F. *et al.* NRF2 activates a partial epithelial-mesenchymal transition and is maximally present in a hybrid epithelial/mesenchymal phenotype. *Integr Biol (Camb)* **11**, 251-263, doi:10.1093/intbio/zyz021 (2019).
358 Chung, V. Y. *et al.* GRHL2-miR-200-ZEB1 maintains the epithelial status of ovarian cancer through transcriptional regulation and histone modification. *Sci Rep* **6**, 19943, doi:10.1038/srep19943 (2016).
359 Jolly, M. K. *et al.* Inflammatory breast cancer: a model for investigating cluster-based dissemination. *NPJ Breast Cancer* **3**, 21, doi:10.1038/s41523-017-0023-9 (2017).
360 Subbalakshmi, A. R., Sahoo, S., Biswas, K. & Jolly, M. K. A Computational Systems Biology Approach Identifies SLUG as a Mediator of Partial Epithelial-Mesenchymal Transition (EMT). *Cells Tissues Organs*, 1-14, doi:10.1159/000512520 (2021).
361 Zander, M. A., Cancino, G. I., Gridley, T., Kaplan, D. R. & Miller, F. D. The Snail transcription factor regulates the numbers of neural precursor cells and newborn neurons throughout mammalian life. *PLoS One* **9**, e104767, doi:10.1371/journal.pone.0104767 (2014).
362 Wang, H. *et al.* ZEB1 Represses Neural Differentiation and Cooperates with CTBP2 to Dynamically Regulate Cell Migration during Neocortex Development. *Cell Rep* **27**, 2335-2353 e2336, doi:10.1016/j.celrep.2019.04.081 (2019).
363 Deryckere, A. *et al.* Multifaceted actions of Zeb2 in postnatal neurogenesis from the ventricular-subventricular zone to the olfactory bulb. *Development* **147**, doi:10.1242/dev.184861 (2020).
364 Di Filippo, E. S. *et al.* Zeb2 Regulates Myogenic Differentiation in Pluripotent Stem Cells. *Int J Mol Sci* **21**, doi:10.3390/ijms21072525 (2020).

365 Choi, I. Y. *et al.* Transcriptional landscape of myogenesis from human pluripotent stem cells reveals a key role of TWIST1 in maintenance of skeletal muscle progenitors. *Elife* **9**, doi:10.7554/eLife.46981 (2020).
366 Gubelmann, C. *et al.* Identification of the transcription factor ZEB1 as a central component of the adipogenic gene regulatory network. *Elife* **3**, e03346, doi:10.7554/eLife.03346 (2014).
367 Goossens, S. *et al.* The EMT regulator Zeb2/Sip1 is essential for murine embryonic hematopoietic stem/progenitor cell differentiation and mobilization. *Blood* **117**, 5620-5630, doi:10.1182/blood-2010-08-300236 (2011).
368 Scott, C. L. & Omilusik, K. D. ZEBs: Novel Players in Immune Cell Development and Function. *Trends Immunol* **40**, 431-446, doi:10.1016/j.it.2019.03.001 (2019).
369 Wang, J. *et al.* Interplay between the EMT transcription factors ZEB1 and ZEB2 regulates hematopoietic stem and progenitor cell differentiation and hematopoietic lineage fidelity. *PLoS Biol* **19**, e3001394, doi:10.1371/journal.pbio.3001394 (2021).
370 Denecker, G. *et al.* Identification of a ZEB2-MITF-ZEB1 transcriptional network that controls melanogenesis and melanoma progression. *Cell Death Differ* **21**, 1250-1261, doi:10.1038/cdd.2014.44 (2014).
371 Bruneel, K., Verstappe, J., Vandamme, N. & Berx, G. Intrinsic Balance between ZEB Family Members Is Important for Melanocyte Homeostasis and Melanoma Progression. *Cancers (Basel)* **12**, doi:10.3390/cancers12082248 (2020).
372 Vandamme, N. & Berx, G. Melanoma cells revive an embryonic transcriptional network to dictate phenotypic heterogeneity. *Front Oncol* **4**, 352, doi:10.3389/fonc.2014.00352 (2014).
373 Goossens, S. *et al.* ZEB2 drives immature T-cell lymphoblastic leukaemia development via enhanced tumour-initiating potential and IL-7 receptor signalling. *Nat Commun* **6**, 5794, doi:10.1038/ncomms6794 (2015).
374 Tang, Y., Durand, S., Dalle, S. & Caramel, J. EMT-Inducing Transcription Factors, Drivers of Melanoma Phenotype Switching, and Resistance to Treatment. *Cancers (Basel)* **12**, doi:10.3390/cancers12082154 (2020).
375 Pedri, D., Karras, P., Landeloos, E., Marine, J. C. & Rambow, F. Epithelial-to-mesenchymal-like transition events in melanoma. *FEBS J* **289**, 1352-1368, doi:10.1111/febs.16021 (2022).
376 Soen, B. *et al.* ZEB Proteins in Leukemia: Friends, Foes, or Friendly Foes? *Hemasphere* **2**, e43, doi:10.1097/HS9.0000000000000043 (2018).
377 Goossens, S. *et al.* ZEB2 and LMO2 drive immature T-cell lymphoblastic leukemia via distinct oncogenic mechanisms. *Haematologica* **104**, 1608-1616, doi:10.3324/haematol.2018.207837 (2019).
378 Vandamme, N. *et al.* The EMT Transcription Factor ZEB2 Promotes Proliferation of Primary and Metastatic Melanoma While Suppressing an Invasive, Mesenchymal-Like Phenotype. *Cancer Res* **80**, 2983-2995, doi:10.1158/0008-5472.CAN-19-2373 (2020).
379 Ishay-Ronen, D. *et al.* Gain Fat-Lose Metastasis: Converting Invasive Breast Cancer Cells into Adipocytes Inhibits Cancer Metastasis. *Cancer Cell* **35**, 17-32 e16, doi:10.1016/j.ccell.2018.12.002 (2019).

Acknowledgements

J.H. is supported by a personal PhD fellowship from FWO (11H9523N). G.B.'s laboratory is supported by the Geconcerteerde Onderzoeksacties Ghent University, Kom Op Tegen Kanker (KoTK) and Stichting tegen Kanker.

Competing interests

The authors declare no competing interests.